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1: J Immunol. 2004 Dec 1;173(11):6928-37. Related Articles, Links



## A novel mechanism for TNF-alpha regulation by p38 MAPK: involvement of NF-kappa B with implications for therapy in rheumatoid arthritis.

Campbell J, Ciesielski CJ, Hunt AE, Horwood NJ, Beech JT, Hayes LA, Denys A, Feldmann M, Brennan FM, Foxwell BM.

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TNF-alpha is a key factor in a variety of inflammatory diseases. This study examines the role of p38 MAPK in the regulation of TNF-alpha in primary human cells relevant to inflammation, e.g., macrophages and rheumatoid synovial cells. Using a dominant negative variant (D168A) of p38 MAPK and a kinase inhibitor, SB203580, we confirm in primary human macrophages that p38 MAPK regulates TNF-alpha production using a posttranscriptional mechanism requiring the 3' untranslated region of the gene. However, in LPS-activated primary human macrophages we also detect a second previously unidentified mechanism, the p38 MAPK modulation of TNF-alpha transcription. This is mediated through p38 MAPK regulation of NF-kappaB. Interestingly this mechanism was not observed in rheumatoid synovial cells. Importantly however, the dominant negative mutant of p38 MAPK, but not SB203580 was effective at inhibiting spontaneous TNF-alpha production in these ex vivo rheumatoid synovial cell cultures. These data indicate there are potential major differences in the role of p38 MAPK in inflammatory signaling that have a bearing on the use of this kinase as a target for therapy. These results indicate despite disappointing results with p38 MAPK inhibitors in the clinic, this kinase is a valid target in rheumatoid disease.

PMID: 15557189 [PubMed - indexed for MEDLINE]

# Anti–Tumor Necrosis Factor-α Antibody Limits Heart Failure in a Transgenic Model

Toshiaki Kadokami, MD, PhD; Carole Frye, MS; Bonnie Lemster, MPH; Carrie L. Wagner, PhD; Arthur M. Feldman, MD, PhD; Charles F. McTiernan, PhD

***Background***—Tumor necrosis factor (TNF)-α has been implicated in the pathophysiology of congestive heart failure. A strain of transgenic mice (TNF1.6) with cardiac-specific overexpression of TNF-α develop congestive heart failure.

***Methods and Results***—To determine the effect of anti–TNF-α therapy in this model, we studied 3 groups: TNF1.6 mice treated with saline, wild-type mice treated with saline, and TNF1.6 mice treated with TNF-α neutralizing antibody (cV1q) from 6 to 12 weeks of age. We used echocardiography to compare cardiac hypertrophy, function, and catecholamine response at 12 weeks of age versus baseline (6 weeks). cV1q treatment did not limit cardiac hypertrophy, but it significantly improved basal fractional shortening and responsiveness to β-adrenergic stimulation, and it limited development of cardiac dilation.

***Conclusions***—Blockade of TNF-α bioactivity by antibody therapy may both preserve cardiac function and partially reverse pathological changes in congestive heart failure. (***Circulation*. 2001;104:1094-1097.**)

**Key Words:** antibodies ■ heart failure ■ hormones

Tumor necrosis factor (TNF)-α may play a pathophysiological role in human heart failure.[1,2] To assess the potential role of TNF-α in heart failure, we generated a transgenic model in which heart failure arises as a consequence of cardiac-specific expression of murine TNF-α.[3] These mice (TNF1.6) display cardiac dilation and fibrosis, loss of cardiac function, reduction of response to β-adrenergic stimulation, increased cardiac infiltrates, enhanced expression of cytokines downstream to TNF-α, reexpression of the fetal gene program, and reduced survival.[3–5] Interestingly, in this model male mice are more severely affected than are female mice.[6]

Anti–TNF-α therapies have proved successful in the treatment of inflammatory diseases, including rheumatoid arthritis and inflammatory bowel diseases. Such therapies may use fusion proteins consisting of soluble TNF receptor and immunoglobulin G (IgG)[7] or antibodies directed against TNF-α.[8,9] Presumably, these agents sequester TNF-α and block interaction with cellular TNF receptors, thereby blunting the biological effects of TNF-α. Previous studies have investigated the utility of receptor-fusion proteins in the treatment of animal models of heart failure.[5,10] In the present study, we investigate the utility of a monoclonal antibody (cV1q) directed against murine TNF-α in modifying cardiac function in the TNF1.6 model of heart failure.

## Methods

Characterizations of TNF1.6 mice have been reported previously.[3–6] Animal use protocols were approved by the Institutional Animal Care and Use Committee. At 6 weeks of age, all mice received echocardiographic assessment. Mice then received either sterile saline (control; both wild-type [WT] and TNF1.6 mice) or the antibody cV1q (IP 0.5 mg/mouse per week; TNF1.6 mice only) for 6 weeks. At 12 weeks of age, mice were again examined by echocardiography. The mice were then euthanatized, and tissues were collected for analysis.

cV1q (Centocor) is a chimeric rat/mouse monoclonal antibody with neutralizing activity against mouse TNF-α. M-mode echocardiographic analyses (baseline and after isoproterenol challenge) were performed as previously described.[4–6] Measurements included left ventricular diastolic dimension (LVDD), left ventricular systolic dimension (LVSD), percentage fractional shortening (%FS), and left ventricular (LV) mass index (LV:body [mg/g]). Cardiac TNF-α and interleukin (IL)-1β levels were measured as previously described.[3,5,6]

Results are reported as mean±SEM. Comparisons between sexes were performed using ANOVA with Student-Newman-Keuls post-hoc tests. Comparisons between mice analyzed at 6 weeks (baseline) and 12 weeks (end of treatment) were performed by paired $t$ tests. Differences were considered significant at $P<0.05$.

## Results

### Echocardiographic Studies

Between 6 and 12 weeks of age, male TNF1.6 mice showed more profound cardiac dysfunction than did female TNF1.6 mice.[6] Age-matched mice were grouped by sex, using male TNF1.6 mice as a model of moderate heart failure and female TNF1.6 mice as a model of mild, progressive heart failure. When grouped by age and sex, 6-week-old WT male mice showed a significantly greater LVDD compared with females (males, 3.89±0.05 mm; females, 3.66±0.05 mm; $P<0.05$).

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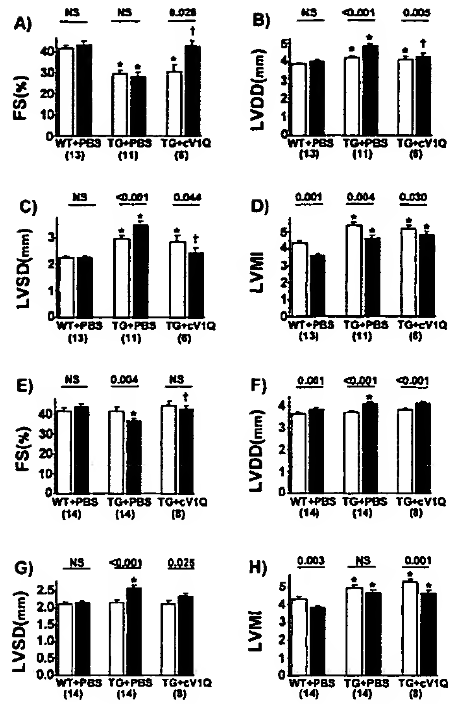

**Figure 1.** Echocardiographic analyses. A-D show results for male mice and E-H, female mice. Open bars represent mice at 6 weeks of age (baseline) and solid bars, 12 weeks of age (end of treatment). TG indicates TNF1.6 mice; PBS, phosphate-buffered saline; and LVMI, left ventricular mass index (mg/g body weight). Number of mice in each group is shown in parentheses. Numbers over bars indicate significance (paired *t* test) between baseline and end of treatment. **P*<0.05 (ANOVA) relative to WT mice of same sex and age; †*P*<0.05 versus TG+PBS.

However, no sex-specific differences in LVSD, %FS, or LV mass index were observed in either 6- or 12-week-old mice, nor were there sex-specific differences in LVDD measured in 12-week-old mice (data not shown). At 6 weeks of age, TNF1.6 males (but not females) showed a significantly reduced basal %FS and increased LVDD and LVSD relative to sex-matched WT mice (Figure 1A-G). Both male and female TNF1.6 mice showed a significant increase in LV mass index relative to sex-matched 6-week-old WT mice (Figure 1C and F), which was not statistically different between the sexes (data not shown).

When treated with saline for 6 weeks (control), male TNF1.6 mice retained an enhanced LV mass index and depressed %FS, whereas cardiac dilation (LVDD and LVSD) significantly increased (Figure 1A-D). Female TNF1.6 mice treated with saline for 6 weeks developed indices of heart failure, as suggested by a significantly reduced %FS and increased measures of cardiac dilation (Figure 1E, F, and H) when compared with either age-matched WT or 6-week-old TNF1.6 female mice.

Treatment with cV1q significantly preserved cardiac function and limited changes in cardiac dilation in both male and female TNF1.6 mice. Thus, %FS in cV1q-treated male mice increased significantly, was significantly higher than that of saline-treated TNF1.6 males, and was equivalent to that of WT males. Both LVDD and LVSD were significantly lower

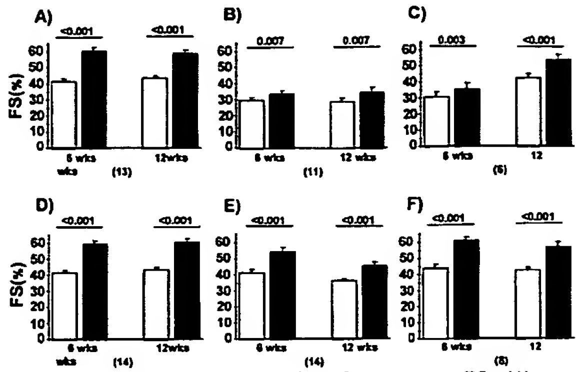


**Figure 2.** Assessment of response to isoproterenol. Response was assessed at baseline (6 wks) and end of treatment (12 wks). Open columns represent measurement before isoproterenol; solid columns, measurement after isoproterenol (300 ng/g body weight IP). A-C show results for male mice and D-F, female mice. A and D show WT+PBS group; B and E, TNF1.6+PBS; and C and F, TNF1.6+cV1q. Number of mice in each group is shown in parentheses. PBS indicates phosphate-buffered saline.

in cV1q-treated males than in saline-treated TNF1.6 males and were equivalent to that of WT males (Figure 1A-C). cV1q-treated females showed a %FS significantly higher than that of saline-treated TNF1.6 females and equivalent to that of age-matched WT females. Cardiac dilation did progress in TNF1.6 females treated with cV1q but was not significantly larger than in age-matched WT females treated with saline, whereas saline-treated TNF1.6 females did show significantly increased LVDD and LVSD (Figure 1E-G). Interestingly, in both male and female TNF1.6 mice, cV1q did not reduce the cardiac hypertrophy already evident at 6 weeks of age when measured by echocardiographic determination of LV mass index (Figure 1D and H) or by gravimetric measure of ventricle mass (data not shown).

Response to β-adrenergic stimulation was also improved in TNF1.6 mice treated with cV1q (Figure 2). Both 6- and 12-week-old male and female WT mice showed similar responses to isoproterenol stimulation, with a marked increase in %FS (Figure 2A and D). Consistent with the more severe heart failure of male TNF1.6, the markedly depressed basal %FS was significantly increased in response to isoproterenol to a much smaller extent in both 6- and 12-week-old male mice (Figure 2B). Female TNF1.6 mice showed a near-normal response to isoproterenol at 6 weeks of age and a significant, although reduced, response at 12 weeks of age (Figure 2E). After treatment with cV1q for 6 weeks, both male and female TNF1.6 mice displayed a significantly increased response to isoproterenol relative to saline-treated TNF1.6 mice and statistically equivalent to that observed in sex-matched saline-treated WT mice (male TNF1.6+saline, 5.6±1.65% [*P*<0.05 relative to other males by ANOVA]; male TNF1.6+cV1q, 11.6±1.42%; male WT+saline, 15.6±1.48%; female TNF1.6+saline, 9.4±1.5% [*P*<0.05 relative to other females by ANOVA]; female TNF1.6+cV1q, 14.3±1.87%; female WT+saline, 17.0±1.40%).

**Myocardial Levels of TNF-$\alpha$ and IL-1$\beta$ in 12-Week-Old TNF1.6 Mice Treated With Saline or cV1q Versus WT Mice Treated With Saline**

| | TNF-$\alpha$ (pg/mg) | IL-1$\beta$ (pg/mg) |
|---|---|---|
| WT+PBS | | |
| M (n=13) | 2.67±0.80 | 4.23±0.64 |
| F (n=11) | 3.34±1.24 | 3.45±1.42 |
| TNF1.6+PBS | | |
| M (n=11) | 286.03±33.0* | 232.3±44.7* |
| F (n=11) | 179.75±28.7* | 163.8±45.3* |
| TNF1.6+cV1q | | |
| M (n=6) | 1350.76±174.1*†‡ | 104.15±22.7 |
| F (n=7) | 819.84±67.2*† | 38.09±7.04 |

M indicates male; F, female; and PBS, phosphate-buffered saline.
*$P<0.05$ vs WT; †$P<0.05$ vs TNF1.6+PBS same sex; ‡$P<0.05$ vs female TNF1.6+cV1q. All comparisons by ANOVA.

## Cardiac Cytokine Expression

At 12 weeks of age, male and female TNF1.6 mice showed significantly elevated myocardial levels of TNF-$\alpha$ and IL-1$\beta$ relative to sex-matched WT mice (Table). In the saline-treated WT and TNF1.6 categories, TNF-$\alpha$ and IL-1$\beta$ levels did not differ between the sexes. After treatment with cV1q, cardiac TNF-$\alpha$ levels significantly increased further in TNF1.6 mice, with the level detected in males significantly greater than that detected in females. Both male and female TNF1.6 mice treated with cV1q demonstrated significantly lower levels of IL-1$\beta$ than did sex-matched TNF1.6 mice treated with saline. Although apparently elevated relative to saline-treated WT mice, the level of myocardial IL-1$\beta$ in cV1q-treated TNF1.6 mice was not significantly different from that of saline-treated WT mice.

# Discussion

Anti–TNF-$\alpha$ therapies have proved effective in the treatment of inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease.[7–9] The elevation of circulating and cardiac levels of TNF-$\alpha$ in human heart failure[1,2,11] and the recapitulation of many aspects of human heart failure in animals models of elevated TNF-$\alpha$ expression[3,10] argue for a possible therapeutic role for anti–TNF-$\alpha$ therapies in heart failure management. Previous animal studies[5,10] suggest that a fusion protein of soluble TNF receptor and IgG may limit biochemical changes underlying heart failure and elicit modest improvements in cardiac function. However, our prior study did not consider the profound differences in the sex-specific progression of heart failure in the TNF1.6 model.[5,6] In the present study, we carefully considered the sex-related differences that occur in the age of onset and severity of heart failure in the TNF1.6 mouse while assessing the use of an anti–TNF-$\alpha$ antibody in treating or preventing heart failure progression.

cV1q is a monoclonal antibody with neutralizing activity against mouse TNF-$\alpha$ and contains rat IgD variable region domains expressed as a fusion protein with murine IgG2a constant domains. In some respects, this antibody resembles cA2 (infliximab), a partially humanized mouse mAb directed against human TNF-$\alpha$, which has been successfully used in the treatment of inflammatory diseases.[8,9] Our previous studies used a recombinant adenovirus in a gene therapy approach to achieve expression of a fusion protein consisting of human soluble TNF receptor I and mouse IgG (sTNFRI-IgG).[4,5] However, serum levels of the fusion protein markedly decreased with time, perhaps because of an immune response to the partially human sequences.

In the present study, we analyzed the sexes separately because treatment of male mice may represent a therapeutic treatment of overt failure, whereas treatment of female mice may resemble a therapy to limit progression. In female TNF1.6 mice, cV1q therapy significantly preserved basal fractional shortening and responses to $\beta$-adrenergic stimulation and partially limited cardiac dilation. These results are more striking than the results in our previous studies with sTNFRI-IgG therapy of TNF1.6 mice, which were performed only on female TNF1.6 mice after 2 weeks of therapy[5] or on 12- or 48-week-old females after 6 weeks of therapy.[4] Both prior studies demonstrated only modest cardiac dysfunction in 8- or 12-week-old female TNF1.6 mice (similar to this report) and either a significant reduction of cardiac dilation (LVSD)[5] or a nonsignificant trend toward preservation of basal fractional shortening.[4,5]

More remarkably, in male TNF1.6 mice in the present study, cV1q therapy significantly improved basal and $\beta$-adrenergic–stimulated cardiac function and reversed or limited progressive cardiac dilation. These novel results were not as apparent in previous studies using female TNF1.6 mice, which display only a modest cardiac dysfunction. This study of male TNF1.6 mice, which demonstrate a much more severe cardiac dysfunction, revealed a marked effect of TNF blockade on both limiting and reversing measures of heart failure and cardiac dilation.

Female mice treated with cV1q in the present study yielded a more notable response than previously observed with sTNFRI-IgG therapy. However, because our prior studies using sTNFRI-IgG therapy did not examine similarly aged male TNF1.6 mice, we can only suggest that cV1q provides greater benefit than sTNFRI-IgG treatment.

An interesting difference between the studies with adenovirus driving overexpression of the sTNFRI-IgG protein and treatment with cV1q antibody is the effect on expression of proinflammatory cytokines in the myocardium. Although both treatments appear to decrease the biological effects of TNF-$\alpha$ while increasing the level of cardiac immunodetectable TNF-$\alpha$, probably through a stabilization of TNF-$\alpha$ protein and prolongation of half-life, the cV1q treatment did not fully normalize the level of immunodetectable IL-1$\beta$, whereas the soluble TNFRI-IgG fusion protein did.[5] These findings are consistent with the observation that the fusion protein decreased the amount of myocardial infiltrates,[5] whereas cV1q therapy did not (data not shown). Whether this represents a fundamental difference in the effect of these 2 therapies is unclear.

Clinical trials in which either anti–TNF-$\alpha$ antibodies or soluble fusion proteins were used to treat inflammatory diseases have yielded mixed results in the attainment of therapeutic benefit. Although both approaches effectively

treat rheumatoid arthritis, anti–TNF-$\alpha$ antibody (cA2) is effective in the treatment of ulcerative colitis,[8] whereas soluble TNF receptor-IgG fusion protein (etanercept) is not effective.[12] Although initial reports on the use of etanercept in human heart failure suggested modest beneficial effects,[13] the clinical trial has recently been terminated for apparent lack of efficacy. However, it would not be without precedent if antibody blockade of TNF-$\alpha$ activity proved more effective than soluble receptor fusion protein in the treatment of congestive heart failure. This report demonstrates that, within the limits of an animal model of heart failure consequent to TNF-$\alpha$ overexpression, therapy with anti–TNF-$\alpha$ antibody can both improve and preserve cardiac function and limit cardiac dilation. Thus, the clinical evaluation of monoclonal anti-TNF therapy in patients with congestive heart failure may be warranted.

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**Note:** Performing your original search, ***tumor necrosis factor is not involved in disease***, in PubMed will retrieve 74820 citations.



1: Chin J Dig Dis. 2005;6(4):170-4. Related Articles, Links

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## Correlation between a gene polymorphism of tumor necrosis factor and inflammatory bowel disease.

Song Y, Wu KC, Zhang L, Hao ZM, Li HT, Zhang LX, Qiao TD, Li CN, Fan DM.

Department of Gastroenterology, Xi'an Municipal Central Hospital, Xi'an, China.

OBJECTIVES: To analyze polymorphism of the tumor necrosis factor (TNF) gene in inflammatory bowel disease (IBD) patients from the Han Chinese ethnic group, and to investigate the role of polymorphism in the pathogenesis of IBD. METHODS: Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) techniques were used to analyze gene polymorphisms in the TNF-alpha and TNF-beta genes in 131 patients with IBD. RESULTS: The genotype frequency and allelic frequency of TNF-alpha-308 in patients with ulcerative colitis (UC) were 15.5% and 8.7%, respectively, significantly higher than the control population (4.1% and 2.0%, respectively; $P < 0.001$). There was no significant difference between patients with Crohn's disease (CD) and the normal population with regard to the genotype frequency and allelic frequency of TNF-alpha-308, and neither were there any differences with regard to TNF-beta+252 in patients with IBD (UC and CD) and the normal population. The TNF-alpha-308 polymorphism and the TNF-beta+252 loci did not correlate with age, gender, disease activity or lesion site for IBD patients. CONCLUSIONS: The TNF-alpha-308 allele may be related to susceptibility to UC. The TNF-alpha-308 gene polymorphism is not involved in pathogenesis of CD. No correlation was found between the TNF-beta+252 polymorphism and IBD. Polymorphisms of the TNF-alpha-308 and TNF-beta+252 loci do not correlate with age, gender, disease activity or lesion site.

PMID: 16246225 [PubMed - indexed for MEDLINE]

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ACCESSION NUMBER: 2004:468813 CAPLUS

DOCUMENT NUMBER: 141:100270

TITLE: Extracellular signal-regulated protein kinase activation is required for metabotropic glutamate receptor-dependent long-term depression in hippocampal area CA1

AUTHOR(S): Gallagher, Sean M.; Daly, Christine A.; Bear, Mark F.; Huber, Kimberly M.

CORPORATE SOURCE: Center for Basic Neuroscience, Department of Physiology, University of Texas Southwestern Medical Center, Dallas, TX, 75390, USA

SOURCE: Journal of Neuroscience (2004), 24(20), 4859-4864
CODEN: JNRSDS; ISSN: 0270-6474

PUBLISHER: Society for Neuroscience

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Activation of group 1 metabotropic glutamate receptors (mGluRs) induces long-term depression (LTD) of synaptic transmission that relies on dendritic protein synthesis. We investigated the signal transduction pathways required for mGluR-LTD to identify candidate mechanisms for mGluR regulation of synaptic protein synthesis. Our results demonstrate a role for extracellular signal-regulated protein kinase (ERK), a subclass of the mitogen-activated protein kinases (MAPKs), in mGluR-LTD in area CA1 of the rat hippocampus. Inhibitors of the upstream kinase of ERK, MAP/ERK kinase significantly reduce mGluR-LTD induced by the group 1 agonist dihydroxyphenylglycine (DHPG) and synaptic stimulation but do not affect NMDA receptor-dependent LTD. In contrast, inhibitors of p38 MAPK were **ineffective** against DHPG-induced LTD. Consistent with the role of ERK in mGluR-LTD, we observed that DHPG treatment of hippocampal slices (isolated CA1), at concns. that induce LTD, results in a robust phosphorylation of ERK but not of p38 MAPK. These results point to ERK as an important regulator of mGluR-LTD and a potential mechanism for mGluR regulation of synaptic protein synthesis.

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L5 ANSWER 32 OF 45 CAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2004:992147 CAPLUS
DOCUMENT NUMBER: 142:153971

TITLE: Characterization of pristane-induced arthritis, a murine model of chronic disease: response to antirheumatic agents, expression of joint cytokines, and immunopathology

AUTHOR(S): Patten, Christopher; Bush, Katherine; Rioja, Inma; Morgan, Rebecca; Wooley, Paul; Trill, John; Life, Paul
CORPORATE SOURCE: GlaxoSmithKline Medicines Research Centre, Stevenage, UK

SOURCE: Arthritis & Rheumatism (2004), 50(10), 3334-3345
CODEN: ARHEAW; ISSN: 0004-3591
PUBLISHER: John Wiley & Sons, Inc.
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Objective. To characterize chronic murine pristane-induced arthritis (PIA) with regard to the response to antirheumatic agents, expression levels of proinflammatory cytokines, and immunopathol. features. Methods. Male DBA/1 mice were injected i.p. with pristane oil to induce a chronic polyarthritis, which was monitored by visual scoring. Serum antibody and splenocyte responses to a panel of putative joint-derived autoantigens were measured. Whole paws were evaluated postmortem for changes in the levels of proinflammatory cytokines tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), and IL-6 by ELISA, anal standard histopathol. techniques were used to determine joint structural changes. Therapeutic studies were performed for up to 8 wk of dosing with prednisolone, methotrexate, 3 nonsteroidal antiinflammatory drugs (celecoxib, diclofenac, and indomethacin), a p38 MAPK inhibitor, SB242235, and human soluble TNF receptor (sTNFR; etanercept) and murine sTNFR fusion proteins. Results. Antibody and cellular responses to the putative joint autoantigens revealed a broad extent of autoimmunity in PIA. TNFα, IL-1β, and IL-6 were all persistently up-regulated in PIA joints. Prednisolone, methotrexate, celecoxib, indomethacin, and SB242235 all significantly reduced the arthritis scores. Etanercept was **ineffective** in reducing the arthritis scores, whereas murine sTNFR produced a significant, but nonsustained, benefit. Only prednisolone significantly reduced the expression of TNFα, IL-1β, and IL-6 in the joints. Prednisolone and methotrexate demonstrated the most effective joint protection. Conclusion. the authors have markedly extended the characterization of PIA as a murine model of chronic inflammatory arthritis by demonstrating cellular and humoral autoantigenicity, elevation of clin. precedented joint cytokines, and variation in the response to several antirheumatic therapies. PIA offers significant potential for the long-term study of immunopathol. mechanisms and novel therapies in rheumatoid arthritis.

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ACCESSION NUMBER: 2002:401264 BIOSIS
DOCUMENT NUMBER: PREV200200401264
TITLE: Synthesis and pharmacological characterization of a potent, orally active **p38** kinase inhibitor.
AUTHOR(S): Dumas, Jacques [Reprint author]; Hatoum-Mokdad, Holia; Sibley, Robert N.; Smith, Roger A.; Scott, William J.; Khire, Uday; Lee, Wendy; Wood, Jill; Wolanin, Donald; Cooley, Jeffrey; Bankston, Donald; Redman, Aniko M.; Schoenleber, Robert; Caringal, Yolanda; Gunn, David; Romero, Romulo; Osterhout, Martin; Paulsen, Holger; Housley, Timothy J.; Wilhelm, Scott M.; Pirro, John; Chien, Du-Shieng; Ranges, Gerald E.; Shrikhande, Alka; Muzsi, Andrew; Bortolon, Elizabeth; Wakefield, Jean; Ostravage, Cynthia Gianpaolo; Bhargava, Ajay; Chau, Thuy
CORPORATE SOURCE: Department of Chemistry Research, Bayer Research Center, 400 Morgan Lane, West Haven, CT, 06516, USA
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SOURCE: Bioorganic and Medicinal Chemistry Letters, (17 June, 2002) Vol. 12, No. 12, pp. 1559-1562. print.
CODEN: BMCLE8. ISSN: 0960-894X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jul 2002
Last Updated on STN: 29 Aug 2002
AB Inhibitors of the MAP kinase **p38** provide a novel approach for the treatment of osteoporosis, inflammatory disorders, and cancer. We have identified N-(3-tert-butyl-1-methyl-5-pyrazolyl)-N'-(4-(4-pyridinylmethyl)phenyl)**urea** as a potent and selective **p38** kinase inhibitor in biochemical and cellular assays. This compound is orally active in two acute models of cytokine release (TNF-induced IL-6 and LPS-induced TNF) and a chronic model of arthritis (20-day murine collagen-induced arthritis).

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Immune System (Chemical Coordination and Homeostasis); Methods and Techniques; Pharmacology; Skeletal System (Movement and Support); Tumor Biology

IT Diseases
arthritis: joint disease, drug therapy
Arthritis (MeSH)

IT Diseases
cancer: neoplastic disease, drug therapy
Neoplasms (MeSH)

IT Diseases
inflammatory disorder: immune system disease, drug therapy

IT Diseases
osteoporosis: bone disease, drug therapy
Osteoporosis (MeSH)

IT Chemicals & Biochemicals
IL-6 [interleukin-6]; LPS [lipopolysaccharide]; N-3(3-tert-butyl-1-methyl-5-pyrazolyl)-N'-(4-(4-pyridinylmethyl)phenyl)**urea**: antiarthritic-drug, antiinflammatory-drug, antineoplastic-drug, enzyme inhibitor-drug, immunologic-drug, antiosteoporotic activity, orally active, pharmacological characterization, synthesis; TNF [**tumor necrosis** factor]; **p38** mitogen-activated protein kinase; **p38** mitogen-activated protein kinase inhibitor: antiarthritic-drug, antiinflammatory-drug, antineoplastic-drug, enzyme inhibitor-drug, immunologic-drug, antiosteoporotic activity, orally active, pharmacological characterization, synthesis

IT Methods & Equipment
chemical synthesis: Synthetic Techniques, pharmacological method, synthetic method

RN 165245-96-5 (**p38** mitogen-activated protein kinase)

STN
ACCESSION NUMBER: 2006:4240 BIOSIS
DOCUMENT NUMBER: PREV200600009251
TITLE: Structure-activity relationships of **p38** mitogen-activated protein kinase inhibitors.
AUTHOR(S): Bolos, Jordi [Reprint Author]
CORPORATE SOURCE: Prous Inst Collaborat Biomed Res, Lab P1C41, Barcelona Sci Pk, Barcelona 08028, Spain
JORDI-BOLOS@terra.es
SOURCE: Mini-Reviews in Medicinal Chemistry, (SEP 2005) Vol. 5, No. 9, pp. 857-868.
ISSN: 1389-5575.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Dec 2005
Last Updated on STN: 14 Dec 2005
AB Rheumatoid arthritis and other chronic inflammatory diseases constitute a major therapeutic challenge, usually not sufficiently met by the classical anti inflammatory medications. Recent research efforts provided new insights into the molecular basis of these pathologies and disclosed new opportunities for developing improved drugs directed to the chemical mediators of the disease. The enzyme **p38** MAP kinase plays a central role in the signal transduction cascade that leads to the production of both the proinflammatory cytokines, TNF-alpha and IL-1 beta, thus representing an attractive therapeutic target for novel antiinflammatory therapies. A number of **p38** inhibitors belonging to different structural families have been developed as potential antiinflammatory drugs, and some of them progressed into clinical trials. The initial pyridinyl imidazole inhibitors contributed to the identification and characterization of **p38** MAP kinase as the molecular target of these new drugs, and were found to act as competitive inhibitors at the ATP binding site of the enzyme. A number of variations in the pyridine and imidazole rings were subsequently introduced. Other inhibitors structurally unrelated to the pyridinylimidazoles have also been developed, such as the pyridopyridazinones, diaryl **ureas**, aminobenzophenones and aromatic amides. One of these structural classes, the N,N'-diarylureas, has been found to interact with a distinct allosteric site of **p38** MAP kinase and requires a deep conformational change prior to binding.

IT Major Concepts
Pharmacology; Rheumatology (Human Medicine, Medical Sciences); Clinical Immunology (Human Medicine, Medical Sciences); Enzymology (Biochemistry and Molecular Biophysics)

IT Diseases
rheumatoid arthritis: immune system disease, joint disease, connective tissue disease, drug therapy
Arthritis, Rheumatoid (MeSH)

IT Diseases
chronic inflammatory disease: immune system disease, drug therapy

IT Chemicals & Biochemicals
**tumor necrosis** factor-alpha; **p38** mitogen-activated protein kinase [EC 2.7.1.37]; interleukin-1-beta; proinflammatory cytokine; ATP: binding; pyridinylimidazoles: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug; pyridine: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug; imidazole: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug; diaryl **ureas**: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug; aminobenzophenones: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug

IT Miscellaneous Descriptors
signal transduction

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human (common)
Taxa Notes

Animals, Chordates, Humans, Mammals, Primates, Vertebrates
RN 165245-96-5 (**p38** mitogen-activated protein kinase)
165245-96-5 (EC 2.7.1.37)
111839-44-2 (ATP)
110-86-1 (pyridine)
288-32-4 (imidazole)

L12 ANSWER 3 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2002344357 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12086485
TITLE: Pyrazole **urea**-based inhibitors of **p38** MAP kinase: from lead compound to clinical candidate.
AUTHOR: Regan John; Breitfelder Steffen; Cirillo Pier; Gilmore Thomas; Graham Anne G; Hickey Eugene; Klaus Bernhard; Madwed Jeffrey; Moriak Monica; Moss Neil; Pargellis Chris; Pav Sue; Proto Alfred; Swinamer Alan; Tong Liang; Torcellini Carol
CORPORATE SOURCE: Department of Medicinal Chemistry, Research and Development Center, Boehringer Ingelheim Pharmaceuticals, 900 Ridgebury Road, Ridgefield, CT 06877, USA.. jregan@rdg.boehringer-ingelheim.com
SOURCE: Journal of medicinal chemistry, (2002 Jul 4) Vol. 45, No. 14, pp. 2994-3008.
Journal code: 9716531. ISSN: 0022-2623.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200208
ENTRY DATE: Entered STN: 28 Jun 2002
Last Updated on STN: 3 Aug 2002
Entered Medline: 2 Aug 2002
AB We report on a series of N-pyrazole, N'-aryl **ureas** and their mode of binding to **p38** mitogen activated protein kinase. Importantly, a key binding domain that is distinct from the adenosine 5'-triphoshate (ATP) binding site is exposed when the conserved activation loop, consisting in part of Asp168-Phe169-Gly170, adopts a conformation permitting lipophilic and hydrogen bonding interactions between this class of inhibitors and the protein. We describe the correlation of the structure-activity relationships and crystallographic structures of these inhibitors with **p38**. In addition, we incorporated another binding pharmacophore that forms a hydrogen bond at the ATP binding site. This modification affords significant improvements in binding, cellular, and in vivo potencies resulting in the selection of 45 (BIRB 796) as a clinical candidate for the treatment of inflammatory diseases.

L12 ANSWER 5 OF 24 BIOSIS 

ACCESSION NUMBER: 2000:466357 BIOSIS
DOCUMENT NUMBER: PREV200000466357
TITLE: 1-Phenyl-5-pyrazolyl **ureas**: Potent and selective **p38** kinase inhibitors.

AUTHOR(S): Dumas, Jacques [Reprint author]; Hatoum-Mokdad, Holia; Sibley, Robert; Riedl, Bernd; Scott, William J.; Monahan, Mary Katherine; Lowinger, Timothy B.; Brennan, Catherine; Natero, Reina; Turner, Tiffany; Johnson, Jeffrey S.; Schoenleber, Robert; Bhargava, Ajay; Wilhelm, Scott M.; Housley, Timothy J.; Ranges, Gerald E.; Shrikhande, Alka
CORPORATE SOURCE: Department of Chemistry Research, Bayer Research Center, 400 Morgan Lane, West Haven, CT, 06516, USA
SOURCE: Bioorganic and Medicinal Chemistry Letters, (18 September, 2000) Vol. 10, No. 18, pp. 2051-2054. print.
CODEN: BMCLE8. ISSN: 0960-894X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Nov 2000
Last Updated on STN: 10 Jan 2002

AB Inhibitors of the MAP kinase **p38** are potentially useful for the treatment of arthritis and osteoporosis. Several 2,3-dichlorophenyl **ureas** were identified as small-molecule inhibitors of **p38** by a combinatorial chemistry effort. Optimization for cellular potency led to the discovery of a new class of potent and selective **p38** kinase inhibitors, exemplified by the 1-phenyl-5-pyrazolyl **urea** 7 (IC50=13 nM).

L12 ANSWER 6 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2005569924 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16247337
TITLE: Role of **p38** mitogen-activated protein kinase on renal dysfunction after hemorrhagic shock in rats.
AUTHOR: Sato Hiroaki; Tanaka Toshiko; Kasai Kentaro; Kita Toshiro; Tanaka Noriyuki
CORPORATE SOURCE: Department of Forensic Medicine, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan.. h-sato@med.uoeh-u.ac.jp
SOURCE: Shock (Augusta, Ga.), (2005 Nov) Vol. 24, No. 5, pp. 488-94.
Journal code: 9421564. ISSN: 1073-2322.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200603
ENTRY DATE: Entered STN: 26 Oct 2005
Last Updated on STN: 21 Mar 2006
Entered Medline: 20 Mar 2006
AB Hemorrhagic shock has been reported to induce renal dysfunction as a consequence of different kinds of local inflammatory response. **p38** mitogen-activated protein kinase (MAPK) is a key mediator in organ dysfunction relating to the inflammatory states, and acts as an important mediator in the intracellular signal pathway for proliferation, differentiation, and production of proinflammatory cytokines such as TNF-alpha and IL-1beta. The effect of **p38** MAPK on the hemorrhagic damage has not been clearly estimated as yet. In this study, our aim was to evaluate the role of **p38** MAPK on the renal damage during the first 5 h after a hemorrhage using a specific inhibitor of **p38** MAPK activation, FR167653. **p38** MAPK activation increased immediately after a hemorrhage and decreased with time. renal mRNA expression of TNF-alpha and IL-1beta increased, renal dysfunction continued to progress, and histological inflammatory injuries were confirmed after hemorrhagic shock. With the pretreatment of FR167653, all of these hemorrhagic changes were attenuated, although the induction of the primary hypotensive state was confirmed. This study demonstrated that renal **p38** MAPK is activated in hemorrhagic shock, promotes the expression of proinflammatory cytokines in the kidney, and consequently develops renal dysfunction. We concluded that **p38** MAPK activation is essential in causing renal damage and that the inhibition of **p38** MAPK activation blocks the development of the renal dysfunction after hemorrhagic shock.

L12 ANSWER 8 OF 24 MEDLINE on STN

ACCESSION NUMBER: 2002182175 MEDLINE

DOCUMENT NUMBER: PubMed ID: 11896401

TITLE: Inhibition of **p38** MAP kinase by utilizing a novel allosteric binding site.

AUTHOR: Pargellis Christopher; Tong Liang; Churchill Laurie; Cirillo Pier F; Gilmore Thomas; Graham Anne G; Grob Peter M; Hickey Eugene R; Moss Neil; Pav Susan; Regan John

CORPORATE SOURCE: Department of Biology, Boehringer Ingelheim Pharmaceuticals, Research and Development Center, 900 Ridgebury Road, Ridgefield, Connecticut 06877, USA.. cpargel@rdg.boehringer-ingelheim.com

SOURCE: Nature structural biology, (2002 Apr) Vol. 9, No. 4, pp. 268-72.
Journal code: 9421566. ISSN: 1072-8368.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: PDB-1KV1; PDB-1KV2

ENTRY MONTH: 200204

ENTRY DATE: Entered STN: 1 Apr 2002
Last Updated on STN: 23 Apr 2002
Entered Medline: 22 Apr 2002

AB The **p38** MAP kinase plays a crucial role in regulating the production of proinflammatory cytokines, such as **tumor necrosis** factor and interleukin-1. Blocking this kinase may offer an effective therapy for treating many inflammatory diseases. Here we report a new allosteric binding site for a diaryl **urea** class of highly potent and selective inhibitors against human **p38** MAP kinase. The formation of this binding site requires a large conformational change not observed previously for any of the protein Ser/Thr kinases. This change is in the highly conserved Asp-Phe-Gly motif within the active site of the kinase. Solution studies demonstrate that this class of compounds has slow binding kinetics, consistent with the requirement for conformational change. Improving interactions in this allosteric pocket, as well as establishing binding interactions in the ATP pocket, enhanced the affinity of the inhibitors by 12,000-fold. One of the most potent compounds in this series, BIRB 796, has picomolar affinity for the kinase and low nanomolar inhibitory activity in cell culture.

L12 ANSWER 9 OF 24 EMBASE 

ACCESSION NUMBER: 2003419444 EMBASE

TITLE: Structure-Activity Relationships of the **p38** α MAP Kinase Inhibitor 1-(5-tert-Butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy) naphthalen-1-yl]**urea** (BIRB 796).

AUTHOR: Regan J.; Capolino A.; Cirillo P.F.; Gilmore T.; Graham A.G.; Hickey E.; Kroe R.R.; Madwed J.; Moriak M.; Nelson R.; Pargellis C.A.; Swinamer A.; Torcellini C.; Tsang M.; Moss N.

CORPORATE SOURCE: J. Regan, Department of Medicinal Chemistry, Boehringer Ingelheim Pharmaceuticals, Research and Development Center, 900 Ridgebury Road, Ridgefield, CT 06877, United States. jregan@rdg.boehringer-ingelheim.com

SOURCE: Journal of Medicinal Chemistry, (23 Oct 2003) Vol. 46, No. 22, pp. 4676-4686. .
Refs: 35
ISSN: 0022-2623 CODEN: JMCMAR

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index

LANGUAGE: English

SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 13 Nov 2003
Last Updated on STN: 13 Nov 2003

AB We report on the structure-activity relationships (SAR) of 1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy) naphthalen-1-yl]**urea** (BIRB 796), an inhibitor of **p38** α MAP kinase which has advanced into human clinical trials for the treatment of autoimmune diseases. Thermal denaturation was used to establish molecular binding affinities for this class of **p38** α inhibitors. The tert-butyl group remains a critical binding element by occupying a lipophilic domain in the kinase which is exposed upon rearrangement of the activation loop. An aromatic ring attached to N-2 of the pyrazole nucleus provides important π-CH (2) interactions with the kinase. The role of groups attached through an ethoxy group to the 4-position of the naphthalene and directed into the ATP-binding domain is elucidated. Pharmacophores with good hydrogen bonding potential, such as morpholine, pyridine, and imidazole, shift the melting temperature of **p38**α by 16-17 °C translating into K(d) values of 50-100 pM. Finally, we describe several compounds that potently inhibit TNF-α production when dosed orally in mice.

L12 ANSWER 20 OF 24 BIOSIS 

ACCESSION NUMBER: 2002:603327 BIOSIS

DOCUMENT NUMBER: PREV200200603327

TITLE: Pyrazole **urea** based **p38** inhibitors: Discovery of a clinical candidate.

AUTHOR(S): Moss, Neil [Reprint author]; Regan, John [Reprint author]; Pargellis, Christopher [Reprint author]; Madwed, Jeff [Reprint author]; Tong, Liang [Reprint author]; Cirillo, Pier [Reprint author]; Hickey, Eugene [Reprint author]; Gilmore, Tom [Reprint author]

CORPORATE SOURCE: Boehringer Ingelheim Pharmaceuticals, Inc, 900 Ridgebury Road, P.O. Box 368, Ridgefield, CT, 06877-0368, USA nmoss@rdg.boehringer-ingelheim.com

SOURCE: Abstracts of Papers American Chemical Society, (2002) Vol. 223, No. 1-2, pp. MEDI 262. print.
Meeting Info.: 223rd National Meeting of the American Chemical Society. Orlando, FL, USA. April 07-11, 2002.
CODEN: ACSRAL. ISSN: 0065-7727.

DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 27 Nov 2002
Last Updated on STN: 27 Nov 2002

L12 ANSWER 21 OF 24 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

| | |
|---|---|
| ACCESSION NUMBER: | 2000:435386 BIOSIS |
| DOCUMENT NUMBER: | PREV200000435386 |
| TITLE: | Pharmacological characterization of pyrazolyl **urea** **p38** kinase inhibitors. |
| AUTHOR(S): | Ranges, Gerald E. [Reprint author]; Bortolon, Elisabeth [Reprint author]; Chau, Thuy [Reprint author]; Dixon, Brian R. [Reprint author]; Bhargava, Ajay [Reprint author]; Dumas, Jacques [Reprint author]; Gianpaolo-Ostravage, Cynthia [Reprint author]; Hatoum-Mokdad, Holia [Reprint author]; Housley, Timothy J. [Reprint author]; Shrikhande, Alka [Reprint author]; Scott, William J. [Reprint author]; Sibley, Robert [Reprint author]; Wakefield, Jean [Reprint author]; Wilhelm, Scott M. [Reprint author] |
| CORPORATE SOURCE: | Bayer Research Center, Pharmaceutical Division, Bayer Corporation, 400, Morgan Lane, West Haven, CT, 06516, USA |
| SOURCE: | Abstracts of Papers American Chemical Society, (2000) Vol. 220, No. Part 1, pp. MEDI 149. print.<br>Meeting Info.: 220th National Meeting of the American Chemical Society. Washington DC, Washington DC, USA. August 20-24, 2000. American Chemical Society.<br>CODEN: ACSRAL. ISSN: 0065-7727. |
| DOCUMENT TYPE: | Conference; (Meeting)<br>Conference; Abstract; (Meeting Abstract) |
| LANGUAGE: | English |
| ENTRY DATE: | Entered STN: 11 Oct 2000<br>Last Updated on STN: 10 Jan 2002 |

L12 ANSWER 5 OF 24 BIOSIS 

ACCESSION NUMBER: 2000:466357 BIOSIS
DOCUMENT NUMBER: PREV200000466357

TITLE: 1-Phenyl-5-pyrazolyl **ureas**: Potent and selective **p38** kinase inhibitors.

AUTHOR(S): Dumas, Jacques [Reprint author]; Hatoum-Mokdad, Holia; Sibley, Robert; Riedl, Bernd; Scott, William J.; Monahan, Mary Katherine; Lowinger, Timothy B.; Brennan, Catherine; Natero, Reina; Turner, Tiffany; Johnson, Jeffrey S.; Schoenleber, Robert; Bhargava, Ajay; Wilhelm, Scott M.; Housley, Timothy J.; Ranges, Gerald E.; Shrikhande, Alka

CORPORATE SOURCE: Department of Chemistry Research, Bayer Research Center, 400 Morgan Lane, West Haven, CT, 06516, USA

SOURCE: Bioorganic and Medicinal Chemistry Letters, (18 September, 2000) Vol. 10, No. 18, pp. 2051-2054. print.
CODEN: BMCLE8. ISSN: 0960-894X.

DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 1 Nov 2000
Last Updated on STN: 10 Jan 2002

AB Inhibitors of the MAP kinase **p38** are potentially useful for the treatment of arthritis and osteoporosis. Several 2,3-dichlorophenyl **ureas** were identified as small-molecule inhibitors of **p38** by a combinatorial chemistry effort. Optimization for cellular potency led to the discovery of a new class of potent and selective **p38** kinase inhibitors, exemplified by the 1-phenyl-5-pyrazolyl **urea** 7 (IC50=13 nM).

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics)

IT Diseases
arthritis: joint disease
Arthritis (MeSH)

IT Diseases
osteoporosis: bone disease
Osteoporosis (MeSH)

IT Chemicals & Biochemicals
1-phenyl-5-pyrazolyl **urea**-7: **p38** kinase inhibitor; 2,3-dichlorophenyl **ureas**: **p38** inhibitors; Il-6 [interleukin-6]; SB203580: MAP kinase **p38** inhibitor; TNF [ **tumor necrosis** factor]; **p38**: MAP kinase

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
SW1353 cell line
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 152121-47-6 (SB203580)

L12 ANSWER 6 OF 24 MEDLINE on STN

ACCESSION NUMBER: 2005569924 MEDLINE

DOCUMENT NUMBER: PubMed ID: 16247337

TITLE: Role of **p38** mitogen-activated protein kinase on renal dysfunction after hemorrhagic shock in rats.

AUTHOR: Sato Hiroaki; Tanaka Toshiko; Kasai Kentaro; Kita Toshiro; Tanaka Noriyuki

CORPORATE SOURCE: Department of Forensic Medicine, School of Medicine, University of Occupational and Environmental Health, Kitakyushu 807-8555, Japan.. h-sato@med.uoeh-u.ac.jp

SOURCE: Shock (Augusta, Ga.), (2005 Nov) Vol. 24, No. 5, pp. 488-94.
Journal code: 9421564. ISSN: 1073-2322.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200603

ENTRY DATE: Entered STN: 26 Oct 2005
Last Updated on STN: 21 Mar 2006
Entered Medline: 20 Mar 2006

AB Hemorrhagic shock has been reported to induce renal dysfunction as a consequence of different kinds of local inflammatory response. **p38** mitogen-activated protein kinase (MAPK) is a key mediator in organ dysfunction relating to the inflammatory states, and acts as an important mediator in the intracellular signal pathway for proliferation, differentiation, and production of proinflammatory cytokines such as TNF-alpha and IL-1beta. The effect of **p38** MAPK on the hemorrhagic damage has not been clearly estimated as yet. In this study, our aim was to evaluate the role of **p38** MAPK on the renal damage during the first 5 h after a hemorrhage using a specific inhibitor of **p38** MAPK activation, FR167653. **p38** MAPK activation increased immediately after a hemorrhage and decreased with time. renal mRNA expression of TNF-alpha and IL-1beta increased, renal dysfunction continued to progress, and histological inflammatory injuries were confirmed after hemorrhagic shock. With the pretreatment of FR167653, all of these hemorrhagic changes were attenuated, although the induction of the primary hypotensive state was confirmed. This study demonstrated that renal **p38** MAPK is activated in hemorrhagic shock, promotes the expression of proinflammatory cytokines in the kidney, and consequently develops renal dysfunction. We concluded that **p38** MAPK activation is essential in causing renal damage and that the inhibition of **p38** MAPK activation blocks the development of the renal dysfunction after hemorrhagic shock.

L12 ANSWER 7 OF 24 EMBASE 
ACCESSION NUMBER: 2005504601 EMBASE
TITLE: Discovery of highly selective inhibitors of **p38** α.
AUTHOR: Popa-Burke I.; Birkos S.; Blackwell L.; Cheatham L.; Clark J.; Dickson Jr. J.K.; Galasinski S.; Janzen W.P.; Mendoza J.; Miller J.L.; Mohney R.P.; Steed P.M.; Hodge C.N.
CORPORATE SOURCE: I. Popa-Burke, Amphora Discovery Corporation, P.O. Box 12169, Research Triangle Park, NJ 27709, United States. Ioana.Popa-Burke@amphoracorp.com
SOURCE: Current Topics in Medicinal Chemistry, (2005) Vol. 5, No. 10, pp. 941-951. .
Refs: 35
ISSN: 1568-0266 CODEN: CTMCCL
COUNTRY: Netherlands
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 28 Nov 2005
Last Updated on STN: 28 Nov 2005
AB The **p38** MAP kinases are a family of serine/threonine protein kinases that play a key role in cellular pathways leading tp pro-inflammatory responses. We have developed and implemented a method for rapidly identifying and optimizing potent and selective **p38** α inhibitors, which is amenable to other targets and target classes. A diverse library of druggable, purified and quantitated molecules was assembled and standardized enzymatic assays were performed in a microfluidic format that provided very accurate and precise inhibition data allowing for development of SAR directly from the primary HTS. All compounds were screened against a collection of more than 60 enzymes (kinases, proteases and phosphatases), allowing for removal of promiscuous and non-selective inhibitors very early in the discovery process. Follow-up enzymological studies included measurement of concentration of compound in buffer, yielding accurate determination of K(i) and IC(50) values, as well as mechanism of action. In addition, active compounds were screened against less desirable properties such as inhibition of the enzyme activity by aggregation, irreversible binding, and time-dependence. Screening of an 88,634-compound library through the above-described process led to the rapid identification of multiple scaffolds (>5 active compounds per scaffold) of potential drug leads for **p38α** that are highly selective against all other enzymes tested, including the three other **p38** isoforms. Potency and selectivity data allowed prioritization of the identified scaffolds for optimization. Herein we present results around our 3-thio-1,2,4-triazole lead series of **p38α** selective inhibitors, including identification, SAR, synthesis, selectivity profile, enzymatic and cellular data in their progression towards drug candidates. .COPYRGT. 2005 Bentham Science Publishers Ltd.

L12 ANSWER 8 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2002182175 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11896401
TITLE: Inhibition of **p38** MAP kinase by utilizing a novel allosteric binding site.
AUTHOR: Pargellis Christopher; Tong Liang; Churchill Laurie; Cirillo Pier F; Gilmore Thomas; Graham Anne G; Grob Peter M; Hickey Eugene R; Moss Neil; Pav Susan; Regan John
CORPORATE SOURCE: Department of Biology, Boehringer Ingelheim Pharmaceuticals, Research and Development Center, 900 Ridgebury Road, Ridgefield, Connecticut 06877, USA.. cpargel@rdg.boehringer-ingelheim.com
SOURCE: Nature structural biology, (2002 Apr) Vol. 9, No. 4, pp. 268-72.
Journal code: 9421566. ISSN: 1072-8368.
PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1KV1; PDB-1KV2
ENTRY MONTH: 200204
ENTRY DATE: Entered STN: 1 Apr 2002
Last Updated on STN: 23 Apr 2002
Entered Medline: 22 Apr 2002

AB The **p38** MAP kinase plays a crucial role in regulating the production of proinflammatory cytokines, such as **tumor necrosis** factor and interleukin-1. Blocking this kinase may offer an effective therapy for treating many inflammatory diseases. Here we report a new allosteric binding site for a diaryl **urea** class of highly potent and selective inhibitors against human **p38** MAP kinase. The formation of this binding site requires a large conformational change not observed previously for any of the protein Ser/Thr kinases. This change is in the highly conserved Asp-Phe-Gly motif within the active site of the kinase. Solution studies demonstrate that this class of compounds has slow binding kinetics, consistent with the requirement for conformational change. Improving interactions in this allosteric pocket, as well as establishing binding interactions in the ATP pocket, enhanced the affinity of the inhibitors by 12,000-fold. One of the most potent compounds in this series, BIRB 796, has picomolar affinity for the kinase and low nanomolar inhibitory activity in cell culture.

L12 ANSWER 9 OF 24 EMBASE 
ACCESSION NUMBER: 2003419444 EMBASE
TITLE: Structure-Activity Relationships of the **p38** α MAP Kinase Inhibitor 1-(5-tert-Butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy) naphthalen-1-yl]**urea** (BIRB 796).
AUTHOR: Regan J.; Capolino A.; Cirillo P.F.; Gilmore T.; Graham A.G.; Hickey E.; Kroe R.R.; Madwed J.; Moriak M.; Nelson R.; Pargellis C.A.; Swinamer A.; Torcellini C.; Tsang M.; Moss N.
CORPORATE SOURCE: J. Regan, Department of Medicinal Chemistry, Boehringer Ingelheim Pharmaceuticals, Research and Development Center, 900 Ridgebury Road, Ridgefield, CT 06877, United States. jregan@rdg.boehringer-ingelheim.com
SOURCE: Journal of Medicinal Chemistry, (23 Oct 2003) Vol. 46, No. 22, pp. 4676-4686. .
Refs: 35
ISSN: 0022-2623 CODEN: JMCMAR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 026 Immunology, Serology and Transplantation
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 13 Nov 2003
Last Updated on STN: 13 Nov 2003

AB We report on the structure-activity relationships (SAR) of 1-(5-tert-butyl-2-p-tolyl-2H-pyrazol-3-yl)-3-[4-(2-morpholin-4-yl-ethoxy) naphthalen-1-yl]**urea** (BIRB 796), an inhibitor of **p38** α MAP kinase which has advanced into human clinical trials for the treatment of autoimmune diseases. Thermal denaturation was used to establish molecular binding affinities for this class of **p38** α inhibitors. The tert-butyl group remains a critical binding element by occupying a lipophilic domain in the kinase which is exposed upon rearrangement of the activation loop. An aromatic ring attached to N-2 of the pyrazole nucleus provides important π-CH (2) interactions with the kinase. The role of groups attached through an ethoxy group to the 4-position of the naphthalene and directed into the ATP-binding domain is elucidated. Pharmacophores with good hydrogen bonding potential, such as morpholine, pyridine, and imidazole, shift the melting temperature of **p38α** by 16-17 °C translating into K(d) values of 50-100 pM. Finally, we describe several compounds that potently inhibit

TNF-α production when dosed orally in mice.

L12 ANSWER 10 OF 24 EMBASE 
ACCESSION NUMBER: 2005265853 EMBASE
TITLE: COPD: Current therapeutic interventions and future approaches.
AUTHOR: Barnes P.J.; Stockley R.A.
CORPORATE SOURCE: P.J. Barnes, National Heart and Lung Institute, Imperial College School of Medicine, Dovehouse St., London SW3 6LY, United Kingdom. p.j.barnes@imperial.ac.uk
SOURCE: European Respiratory Journal, (2005) Vol. 25, No. 6, pp. 1084-1106. .
Refs: 295
ISSN: 0903-1936 CODEN: ERJOEI
COUNTRY: Switzerland
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 006 Internal Medicine
015 Chest Diseases, Thoracic Surgery and Tuberculosis
030 Pharmacology
037 Drug Literature Index
038 Adverse Reactions Titles
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 30 Jun 2005
Last Updated on STN: 30 Jun 2005

AB Although long-acting bronchodilators have been an important advance for the management of chronic obstructive pulmonary disease (COPD), these drugs do not deal with the underlying inflammatory process. No currently available treatments reduce the progression of COPD or suppress the inflammation in small airways and lung parenchyma. Several new treatments that target the inflammatory process are now in clinical development. Some therapies, such as chemokine antagonists, are directed against the influx of inflammatory cells into the airways and lung parenchyma that occurs in COPD, whereas others target inflammatory cytokines such as tumour necrosis factor-α. Broad spectrum anti-inflammatory drugs are now in phase III development for COPD, and include phosphodiesterase-4 inhibitors. Other drugs that inhibit cell signalling include inhibitors of **p38** mitogen-activated protein kinase, nuclear factor-κB and phosphoinositide-3 kinase-γ. More specific approaches are to give antioxidants, inhibitors of inducible nitric oxide synthase and leukotriene B(4) antagonists. Other treatments have the potential to combat mucus hypersecretion, and there is also a search for serine proteinase and matrix metalloproteinase inhibitors to prevent lung destruction and the development of emphysema. More research is needed to understand the cellular and molecular mechanisms of chronic obstructive pulmonary disease and to develop biomarkers and monitoring techniques to aid the development of new therapies. Copyright .COPYRGT. ERS Journals Ltd 2005.

L12 ANSWER 11 OF 24 EMBASE 
ACCESSION NUMBER: 2004219447 EMBASE
TITLE: Nuclear export inhibitors and kinase inhibitors identified using a MAPK-activated protein kinase 2 redistribution® screen.
AUTHOR: Almholt D.L.C.; Loechel F.; Nielsen S.J.; Krog-Jensen C.; Terry R.; Bjorn S.P.; Pedersen H.C.; Praestegaard M.; Moller S.; Heide M.; Pagliaro L.; Mason A.J.; Butcher S.; Dahl S.W.
CORPORATE SOURCE: S.W. Dahl, BioImage A/S, Morkhoj Bygade 28, DK-2860 Soborg, Denmark. Soeren.Weis.Dahl@bioimage.com
SOURCE: Assay and Drug Development Technologies, (2004) Vol. 2, No. 1, pp. 7-20. .
Refs: 37
ISSN: 1540-658X CODEN: ADDTAR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 027 Biophysics, Bioengineering and Medical

Instrumentation
029 Clinical Biochemistry
030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 4 Jun 2004
Last Updated on STN: 4 Jun 2004

AB Redistribution® (BioImage® A/S, Soborg, Denmark) is a novel high-throughput screening technology that monitors translocation of specific protein components of intracellular signaling pathways within intact mammalian cells, using green fluorescent protein as a tag. A single Redistribution assay can be used to identify multiple classes of compounds that act at, or upstream of, the level of the protein target used in the primary screening assay. Such compounds may include both conventional and allosteric enzyme inhibitors, as well as protein-protein interaction modulators. We have developed a series of Redistribution assays to discover and characterize compounds that inhibit **tumor necrosis** factor-α biosynthesis via modulation of the **p38** mitogen-activated protein kinase (MAPK) pathway. A primary assay was designed to identify low-molecular-weight compounds that inhibit the activation-dependent nuclear export of the **p38** kinase substrate MAPK-activated protein kinase 2 (MK2). Hits from the primary screen were categorized, using secondary assays, either as direct inhibitors of MK2 nuclear export, or as inhibitors of the upstream **p38** MAPK pathway. Activity profiles are presented for a nuclear export inhibitor, and a compound that structurally and functionally resembles a known **p38** kinase inhibitor. These results demonstrate the utility of Redistribution technology as a pathway screening method for the identification of diverse and novel compounds that are active within therapeutically important signaling pathways.


L12 ANSWER 12 OF 24 EMBASE 
ACCESSION NUMBER: 2004017273 EMBASE
TITLE: A novel Pd-catalyzed cyclization reaction of **ureas** for the synthesis of dihydroquinazolinone **p38** kinase inhibitors.
AUTHOR: Schlapbach A.; Heng R.; Di Padova F.
CORPORATE SOURCE: A. Schlapbach, Novartis Inst. Biomed. Res., A., Lichtstrasse, CH-4002 Basel, Switzerland.
achim.schlapbach@pharma.novartis.com
SOURCE: Bioorganic and Medicinal Chemistry Letters, (2004) Vol. 14, No. 2, pp. 357-360. .
Refs: 22
ISSN: 0960-894X CODEN: BMCLE8
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 29 Jan 2004
Last Updated on STN: 29 Jan 2004

AB A series of potent **p38** inhibitors based on the dihydroquinazoline scaffold was synthesized using a novel Pd-catalyzed cyclization reaction of aryl-benzyl **ureas**. Optimization of this compound class led to compound 20, which inhibits **p38α** in vitro with IC(50)=14 nM and is active in the mouse TNFα-release model. 

L12 ANSWER 13 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2002075965 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11801680
TITLE: Hypertonic preconditioning inhibits macrophage responsiveness to endotoxin.
AUTHOR: Cuschieri Joseph; Gourlay David; Garcia Iris; Jelacic Sandra; Maier Ronald V

CORPORATE SOURCE: Department of Surgery, University of Washington, 325 Ninth Avenue, Seattle, WA 98104, USA.. jcuschie@u.washington.edu
CONTRACT NUMBER: GM 45873-08 (NIGMS)
SOURCE: Journal of immunology (Baltimore, Md. : 1950), (2002 Feb 1) Vol. 168, No. 3, pp. 1389-96.
Journal code: 2985117R. ISSN: 0022-1767.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 200202
ENTRY DATE: Entered STN: 25 Jan 2002
Last Updated on STN: 13 Feb 2002
Entered Medline: 12 Feb 2002
AB Hypertonic saline has been shown to modulate cell shape and the response of components of the innate immune response. However, the effect of hypertonic saline on the macrophage remains unknown. We hypothesized that hypertonic preconditioning would impair subsequent inflammatory mediator signaling through a reduction in stress fiber polymerization and mitogen-activated protein kinase activity after LPS stimulation. Rabbit alveolar macrophages were stimulated with 100 ng/ml of LPS. Selected cells were preconditioned with 40-100 mM of NaCl, mannitol, or **urea** for 4 h and returned to isotonic medium before LPS stimulation. Cellular protein was harvested and subjected to Western blot analysis for the dually phosphorylated active forms of **p38** and extracellular signal-related kinase (ERK) 1/2. TNF production was determined by an L929 bioassay, and stress fiber polymerization was evaluated by confocal microscopy. Preconditioning of macrophages with NaCl or mannitol resulted in dose-dependent reduction in ERK 1/2 phosphorylation with no effect on **p38** phosphorylation. **Urea** preconditioning had no effect on either mitogen-activated protein kinase. A dose-dependent attenuation of TNF production was seen with NaCl and mannitol preconditioning ($p < 0.05$), but not with **urea**. NaCl and mannitol preconditioning resulted in failure of LPS-induced stress fiber polymerization, whereas **urea** did not. Extracellular hypertonic conditions (i.e., NaCl and mannitol) have an immunomodulatory effect on macrophages, demonstrated through failure of optimal stress fiber polymerization, ERK 1/2 activity, and TNF production. Intracellular hypertonic conditions (i.e., **urea**) had no significant effect. Hypertonic saline or mannitol resuscitation, therefore, may help protect against multiple-organ dysfunction syndrome as a result of this reduced proinflammatory responsiveness.

L12 ANSWER 14 OF 24 BIOSIS 
ACCESSION NUMBER: 2002:401264 BIOSIS
DOCUMENT NUMBER: PREV200200401264
TITLE: Synthesis and pharmacological characterization of a potent, orally active **p38** kinase inhibitor.
AUTHOR(S): Dumas, Jacques [Reprint author]; Hatoum-Mokdad, Holia; Sibley, Robert N.; Smith, Roger A.; Scott, William J.; Khire, Uday; Lee, Wendy; Wood, Jill; Wolanin, Donald; Cooley, Jeffrey; Bankston, Donald; Redman, Aniko M.; Schoenleber, Robert; Caringal, Yolanda; Gunn, David; Romero, Romulo; Osterhout, Martin; Paulsen, Holger; Housley, Timothy J.; Wilhelm, Scott M.; Pirro, John; Chien, Du-Shieng; Ranges, Gerald E.; Shrikhande, Alka; Muzsi, Andrew; Bortolon, Elizabeth; Wakefield, Jean; Ostravage, Cynthia Gianpaolo; Bhargava, Ajay; Chau, Thuy
CORPORATE SOURCE: Department of Chemistry Research, Bayer Research Center, 400 Morgan Lane, West Haven, CT, 06516, USA
jacques.dumas.b@bayer.com
SOURCE: Bioorganic and Medicinal Chemistry Letters, (17 June, 2002) Vol. 12, No. 12, pp. 1559-1562. print.
CODEN: BMCLE8. ISSN: 0960-894X.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 24 Jul 2002
Last Updated on STN: 29 Aug 2002

AB Inhibitors of the MAP kinase **p38** provide a novel approach for the treatment of osteoporosis, inflammatory disorders, and cancer. We have identified N-(3-tert-butyl-1-methyl-5-pyrazolyl)-N'-(4-(4-pyridinylmethyl)phenyl)**urea** as a potent and selective **p38** kinase inhibitor in biochemical and cellular assays. This compound is orally active in two acute models of cytokine release (TNF-induced IL-6 and LPS-induced TNF) and a chronic model of arthritis (20-day murine collagen-induced arthritis).

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Immune System (Chemical Coordination and Homeostasis); Methods and Techniques; Pharmacology; Skeletal System (Movement and Support); Tumor Biology

IT Diseases
arthritis: joint disease, drug therapy
Arthritis (MeSH)

IT Diseases
cancer: neoplastic disease, drug therapy
Neoplasms (MeSH)

IT Diseases
inflammatory disorder: immune system disease, drug therapy

IT Diseases
osteoporosis: bone disease, drug therapy
Osteoporosis (MeSH)

IT Chemicals & Biochemicals
IL-6 [interleukin-6]; LPS [lipopolysaccharide]; N-3(3-tert-butyl-1-methyl-5-pyrazolyl)-N'-(4-(4-pyridinylmethyl)phenyl)**urea**: antiarthritic-drug, antiinflammatory-drug, antineoplastic-drug, enzyme inhibitor-drug, immunologic-drug, antiosteoporotic activity, orally active, pharmacological characterization, synthesis; TNF [**tumor necrosis** factor]; **p38** mitogen-activated protein kinase; **p38** mitogen-activated protein kinase inhibitor: antiarthritic-drug, antiinflammatory-drug, antineoplastic-drug, enzyme inhibitor-drug, immunologic-drug, antiosteoporotic activity, orally active, pharmacological characterization, synthesis

IT Methods & Equipment
chemical synthesis: Synthetic Techniques, pharmacological method, synthetic method

RN 165245-96-5 (**p38** mitogen-activated protein kinase)

L12 ANSWER 15 OF 24 BIOSIS COPYRIGHT (c) 2006 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:4240 BIOSIS
DOCUMENT NUMBER: PREV200600009251
TITLE: Structure-activity relationships of **p38** mitogen-activated protein kinase inhibitors.
AUTHOR(S): Bolos, Jordi [Reprint Author]
CORPORATE SOURCE: Prous Inst Collaborat Biomed Res, Lab P1C41, Barcelona Sci Pk, Barcelona 08028, Spain
JORDI-BOLOS@terra.es
SOURCE: Mini-Reviews in Medicinal Chemistry, (SEP 2005) Vol. 5, No. 9, pp. 857-868.
ISSN: 1389-5575.
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 14 Dec 2005
Last Updated on STN: 14 Dec 2005

AB Rheumatoid arthritis and other chronic inflammatory diseases constitute a major therapeutic challenge, usually not sufficiently met by the classical anti inflammatory medications. Recent research efforts provided new insights into the molecular basis of these pathologies and disclosed new opportunities for developing improved drugs directed to the chemical mediators of the disease. The enzyme **p38** MAP kinase plays a central role in the signal transduction cascade that leads to the production of both the proinflammatory cytokines, TNF-alpha and IL-1 beta, thus representing an attractive therapeutic target for novel antiinflammatory therapies. A number of **p38** inhibitors belonging to different structural families have been developed as potential antiinflammatory drugs, and some of them progressed into

clinical trials. The initial pyridinyl imidazole inhibitors contributed to the identification and characterization of **p38** MAP kinase as the molecular target of these new drugs, and were found to act as competitive inhibitors at the ATP binding site of the enzyme. A number of variations in the pyridine and imidazole rings were subsequently introduced. Other inhibitors structurally unrelated to the pyridinylimidazoles have also been developed, such as the pyridopyridazinones, diaryl **ureas**, aminobenzophenones and aromatic amides. One of these structural classes, the N,N'-diarylureas, has been found to interact with a distinct allosteric site of **p38** MAP kinase and requires a deep conformational change prior to binding.

IT Major Concepts
Pharmacology; Rheumatology (Human Medicine, Medical Sciences); Clinical Immunology (Human Medicine, Medical Sciences); Enzymology (Biochemistry and Molecular Biophysics)

IT Diseases
rheumatoid arthritis: immune system disease, joint disease, connective tissue disease, drug therapy
Arthritis, Rheumatoid (MeSH)

IT Diseases
chronic inflammatory disease: immune system disease, drug therapy

IT Chemicals & Biochemicals
**tumor necrosis** factor-alpha; **p38** mitogen-activated protein kinase [EC 2.7.1.37]; interleukin-1-beta; proinflammatory cytokine; ATP: binding; pyridinylimidazoles: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug; pyridine: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug; imidazole: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug; diaryl **ureas**: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug; aminobenzophenones: enzyme inhibitor-drug, immunologic-drug, antiinflammatory-drug

IT Miscellaneous Descriptors
signal transduction

ORGN Classifier
Hominidae 86215
Super Taxa
Primates; Mammalia; Vertebrata; Chordata; Animalia
Organism Name
human (common)
Taxa Notes
Animals, Chordates, Humans, Mammals, Primates, Vertebrates

RN 165245-96-5 (**p38** mitogen-activated protein kinase)
165245-96-5 (EC 2.7.1.37)
111839-44-2 (ATP)
110-86-1 (pyridine)
288-32-4 (imidazole)

L12 ANSWER 16 OF 24 EMBASE 

ACCESSION NUMBER: 2005045715 EMBASE
TITLE: Identification of novel **p38**α MAP kinase inhibitors using fragment-based lead generation.
AUTHOR: Gill A.L.; Frederickson M.; Cleasby A.; Woodhead S.J.; Carr M.G.; Woodhead A.J.; Walker M.T.; Congreve M.S.; Devine L.A.; Tisi D.; O'Reilly M.; Seavers L.C.A.; Davis D.J.; Curry J.; Anthony E.; Padova A.; Murray C.W.; Carr R.A.E.; Jhoti H.
CORPORATE SOURCE: A.L. Gill, Astex Technology, Milton Road, Cambridge, CB4 0QA, United Kingdom. a.gill@astex-technology.com
SOURCE: Journal of Medicinal Chemistry, (27 Jan 2005) Vol. 48, No. 2, pp. 414-426. .
Refs: 42
ISSN: 0022-2623 CODEN: JMCMAR
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 030 Pharmacology
037 Drug Literature Index
LANGUAGE: English
SUMMARY LANGUAGE: English

ENTRY DATE: Entered STN: 10 Feb 2005
Last Updated on STN: 10 Feb 2005

AB We describe the structure-guided optimization of the molecular fragments 2-amino-3-benzyl-oxypyridine 1 (IC(50) 1.3 mM) and 3-(2-(4-pyridyl) ethyl)indole 2 (IC(50) 35 μM) identified using X-ray crystallographic screening of **p38α** MAP kinase. Using two separate case studies, the article focuses on the key compounds synthesized, the structure-activity relationships and the binding mode observations made during this optimization process, resulting in two potent lead series that demonstrate significant increases in activity. We describe the process of compound elaboration either through the growing out from fragments into adjacent pockets or through the conjoining of overlapping fragments and demonstrate that we have exploited the mobile conserved activation loop, consisting in part of Asp168-Phe169-Gly170 (DFG), to generate significant improvements in potency and kinase selectivity.

L12 ANSWER 17 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2005646213 MEDLINE
DOCUMENT NUMBER: PubMed ID: 16283677
TITLE: Small molecular anti-cytokine agents.
AUTHOR: Wagner Gerd; Laufer Stefan
CORPORATE SOURCE: School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich, NR4 7TJ, England.
SOURCE: Medicinal research reviews, (2006 Jan) Vol. 26, No. 1, pp. 1-62. Ref: 146
Journal code: 8103150. ISSN: 0198-6325.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200602
ENTRY DATE: Entered STN: 6 Dec 2005
Last Updated on STN: 18 Feb 2006
Entered Medline: 17 Feb 2006

AB The recent successful introduction of the anti-cytokine biologicals Etanercept, Infliximab, Adalimumab, and Anakinra has stimulated the search for anti-cytokine small-molecules. A number of molecular targets have been identified for the development of such small molecular anti-cytokine agents. The focus of this review will be on those inhibitors of cytokine production, which target either **p38** mitogen activated protein (MAP) kinase, TNF-alpha converting enzyme (TACE), or IL-1beta converting enzyme (ICE). **P38** MAP kinase occupies a central role in the signaling network responsible for the upregulation of proinflammatory cytokines like interleukin 1beta (IL-1beta) and TNF-alpha, and regulates their biosynthesis at both the transcriptional and translational level. TACE and ICE are two proteases required for the processing of proTNF-alpha and proIL-1beta, respectively into their mature, proinflammatory form. Since the mid-1990s, a plethora of inhibitors of **p38** MAP kinase, TACE, and ICE has been characterized in vitro, and individual representatives from all three inhibitor classes have in the meantime been advanced into clinical trials. This review will highlight the correlation between effective inhibition at the molecular target and cellular activity in functional assays of cytokine, particularly TNF-alpha and IL-1beta, production. Structure-activity relationships (SAR) will be discussed regarding activity at the respective enzyme target, but also with regard to properties required for efficient in vitro and in vivo cellular activity (e.g., oral availability, solubility, cell penetration, etc.).

L12 ANSWER 18 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2005204575 IN-PROCESS
DOCUMENT NUMBER: PubMed ID: 15836823
TITLE: Protective effect of **p38** mitogen activated protein kinase inhibitor on organs in sepsis in rats.
AUTHOR: Ma Zhong-fu; Le Sheng; Liang Yan-bing; Zhan Hong; Tang Hao; Jing Xiao-li
CORPORATE SOURCE: Emergency Department, First Affiliated Hospital, Sun Yat-sen University, Guangzhou 510080, Guangdong, China.. ma_zf@163.net

SOURCE: Zhongguo wei zhong bing ji jiu yi xue = Chinese critical care medicine = Zhongguo weizhongbing jijiuyixue, (2005 Apr) Vol. 17, No. 4, pp. 211-3.
Journal code: 9887521. ISSN: 1003-0603.
PUB. COUNTRY: China
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: Chinese
FILE SEGMENT: NONMEDLINE; IN-PROCESS; NONINDEXED; Priority Journals
ENTRY DATE: Entered STN: 20 Apr 2005
Last Updated on STN: 14 Dec 2005

AB OBJECTIVE: To investigate the pathogenesis of multiorgan injury and the protective of **p38** mitogen activated protein kinase(p38MAPK) inhibitor on organs in sepsis. METHODS: Cecal ligation and puncture was adopted to reproduce sepsis model. The levels of serum biochemical parameters [including alanine aminotransferase (ALT), blood **urea** nitrogen(BUN), creatinine(Cr), MB isoenzyme of creatine phosphokinase (CPK-MB), **tumor necrosis** factor-alpha(TNF-alpha) and interleukin-1beta(IL-1beta) were determined at different time points. RESULTS:The levels of ALT, BUN, Cr, CPK-MB, TNF-alpha and IL-beta rose progressively after the cecal ligation operation. The levels of TNF-alpha and IL-1beta showed a significant correlation with levels of ALT, BUN, Cr, CPK-MB. After the administration of p38MAPK inhibitor, SB203580, the level of TNF-alpha and IL-1beta were found to decrease evidently, and the injury to multiple organs was alleviated. CONCLUSION: Excessive secretion of TNF-alpha and IL-beta may be the main cause of multiorgan injury in sepsis. Modulation of the p38MAPK pathway may protect multiorgan injury in sepsis.

L12 ANSWER 19 OF 24 MEDLINE on STN
ACCESSION NUMBER: 2005042050 MEDLINE
DOCUMENT NUMBER: PubMed ID: 15389886
TITLE: Sodium 4-phenylbutyrate induces apoptosis of human lung carcinoma cells through activating JNK pathway.
AUTHOR: Zhang Xing; Wei Lin; Yang Yu; Yu Qiang
CORPORATE SOURCE: Pulmonary Center, Department of Medicine, Boston University Medical Center, Boston, Massachusetts 02118, USA.
CONTRACT NUMBER: R01 GM59678 (NIGMS)
SOURCE: Journal of cellular biochemistry, (2004 Nov 1) Vol. 93, No. 4, pp. 819-29.
Journal code: 8205768. ISSN: 0730-2312.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200507
ENTRY DATE: Entered STN: 27 Jan 2005
Last Updated on STN: 15 Jul 2005
Entered Medline: 14 Jul 2005

AB Sodium 4-phenylbutyrate (PB) has been used in the therapy of **urea** cycle defects for many years. Recently, it has been shown to cause cellular differentiation, growth arrest, and apoptosis in certain malignancies. We have analyzed the effects of PB on human lung carcinoma cells. PB has distinct patterns of effects on different lung carcinoma cells, inducing apoptosis in NCI-H460 and NCI-H1792 cells, causing G1 arrest in A549 and SK-LU-1 cells, but having no effect on a non-transformed bronchial epithelial cell line HBE4-E6/E7. We investigated the role of MAP kinase family members, extracellular signal-regulated kinase (ERK), JNK, and **p38** mitogen-activated protein kinase (MAPK), as well as other important cell survival signaling molecules in PB-induced apoptosis. We observed activation of JNK and ERK by PB in the lung cancer cells. JNK was activated only in the two apoptotic cells, whereas ERK was activated in both the apoptotic and the growth-arrested cells, demonstrating a correlation between apoptosis and activation of JNK in response to PB. Both JNK inhibitor and JNK RNA interference (RNAi) inhibited PB-induced apoptosis, whereas MEK inhibitor did not, supporting that apoptosis induced by PB is through activation of JNK. De novo protein synthesis is required for the PB-induced JNK activation and induction of apoptosis. However, the production of known upstream activators of JNK, namely Fas/Fas ligand, **tumor**

**necrosis** factor (TNF)-alpha, TNF-beta, and TRAIL, are not altered by PB treatment. Therefore, PB activates JNK through an unidentified and cell type-specific mechanism. Understanding of this mechanism is of therapeutic value in treating cancer patients with PB.

L12 ANSWER 20 OF 24 BIOSIS 

ACCESSION NUMBER: 2002:603327 BIOSIS
DOCUMENT NUMBER: PREV200200603327
TITLE: Pyrazole **urea** based **p38** inhibitors: Discovery of a clinical candidate.
AUTHOR(S): Moss, Neil [Reprint author]; Regan, John [Reprint author]; Pargellis, Christopher [Reprint author]; Madwed, Jeff [Reprint author]; Tong, Liang [Reprint author]; Cirillo, Pier [Reprint author]; Hickey, Eugene [Reprint author]; Gilmore, Tom [Reprint author]
CORPORATE SOURCE: Boehringer Ingelheim Pharmaceuticals, Inc, 900 Ridgebury Road, P.O. Box 368, Ridgefield, CT, 06877-0368, USA nmoss@rdg.boehringer-ingelheim.com
SOURCE: Abstracts of Papers American Chemical Society, (2002) Vol. 223, No. 1-2, pp. MEDI 262. print.
Meeting Info.: 223rd National Meeting of the American Chemical Society. Orlando, FL, USA. April 07-11, 2002.
CODEN: ACSRAL. ISSN: 0065-7727.
DOCUMENT TYPE: Conference; (Meeting)
Conference; Abstract; (Meeting Abstract)
LANGUAGE: English
ENTRY DATE: Entered STN: 27 Nov 2002
Last Updated on STN: 27 Nov 2002

IT Major Concepts
Pharmacology

IT Diseases
arthritis: joint disease
Arthritis (MeSH)

IT Diseases
inflammatory disease: immune system disease

IT Chemicals & Biochemicals
TNF [**tumor necrosis** factor]; **p38**;
pyrazole **urea**: antiarthritic-drug, antiinflammatory-drug, enzyme inhibitor-drug, immunologic-drug

IT Miscellaneous Descriptors
drug development; Meeting Abstract

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=> d his

     (FILE 'HOME' ENTERED AT 14:21:07 ON 19 MAY 2006)

     FILE 'MEDLINE, BIOSIS, EMBASE' ENTERED AT 14:23:52 ON 19 MAY 2006
L1            0 S 432050-17-4/RN
L2          221 S DUMAS J/AU
L3            0 S L2 AND  RIEDL
L4            0 S L2 AND HAMDEN
L5            0 S DUMAS AND RIEDL
L6            0 S DUMAS AND LOWINGER
L7            0 S KHIRE AND RIEDL
L8            0 S DUMAS AND P38
L9          186 S P38 AND UREA
L10          32 S L9 AND TUMOR NECROSIS
L11          24 DUP REM L10 (8 DUPLICATES REMOVED)
L12          24 FOCUS L11 1-

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L13           1 S 152121-47-6/RN
                SET NOTICE 1 DISPLAY
                SET NOTICE LOGIN DISPLAY

=>
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Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 9–12

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

# p38 Kinase Inhibitors for the Treatment of Arthritis and Osteoporosis: Thienyl, Furyl, and Pyrrolyl Ureas

Anikó M. Redman,[a] Jeffrey S. Johnson,[a] Robert Dally,[a] Steve Swartz,[a] Hanno Wild,[a] Holger Paulsen,[a] Yolanda Caringal,[a] David Gunn,[a] Joel Renick,[a] Martin Osterhout,[a] Jill Kingery-Wood,[a] Roger A. Smith,[a] Wendy Lee,[a] Jacques Dumas,[a] Scott M. Wilhelm,[b] Timothy J. Housley,[b] Ajay Bhargava,[b] Gerald E. Ranges,[b] Alka Shrikhande,[b] Deborah Young,[b] Michael Bombara[b] and William J. Scott[a,*]

[a]*Department of Chemistry Research, Bayer Research Center, Pharmaceutical Division, 400 Morgan Lane, West Haven, CT 06516, USA*

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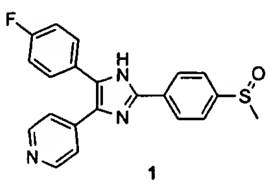
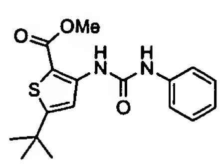
Received 15 August 2000; accepted 2 October 2000

**Abstract**—Inhibitors of the MAP kinase p38 are potentially useful for the treatment for osteoporosis, arthritis, and other inflammatory diseases. A series of thienyl, furyl, and pyrrolyl ureas has been identified as potent p38 inhibitors, displaying in vitro activity in the nanomolar range. 

Members of the MAP kinase family are implicated in the activation of a wide variety of transcription factors and proteins involved in the control of cytokine production. A pair of novel protein kinase homologues (p38) involved in the regulation of cytokine synthesis have been described.[1] Small molecule inhibitors of p38, such as SB 203580 **1**[2,3] (Fig. 1), can potentially lead to the treatment of osteoporosis and inflammatory disorders.




**1**
SB 203580
p38 $IC_{50}$ = 20 nM



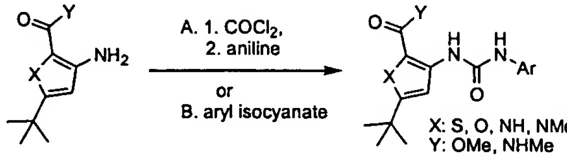

**2**
p38 $IC_{50}$ = 290 nM

**Figure 1.** p38 Kinase inhibitors.

Following high throughput screening of the Bayer compound library, the commercially available thienyl urea **2** (Maybridge GK 00687) was identified as a reversible p38 inhibitor (Fig. 1). It was rapidly shown that the corresponding furans and pyrroles were also active. This paper describes our effort to optimize substitutions on both rings of the lead urea.[4]

## Chemistry

Ureas were synthesized by the reaction of the heterocyclic amines with phosgene (or a phosgene equivalent), followed by treatment with anilines (Scheme 1). Alternatively, heterocyclic amines were reacted with isocyanates.



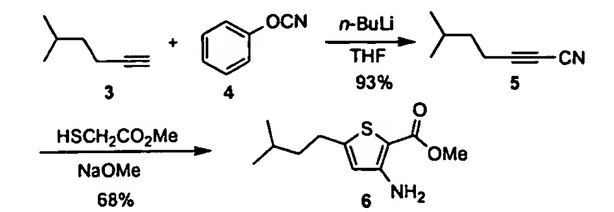

**Scheme 1.** Synthesis of thienyl, furyl, and pyrrole ureas.

In the case of pyrroles, the ring nitrogen did not need protection during this reaction sequence. Methyl 3-amino-

*Corresponding author. Tel.: +1-203-812-2935; fax: +1-203-812-3655; e-mail: william.scott.b@bayer.com, aniko.redman.b@bayer.com

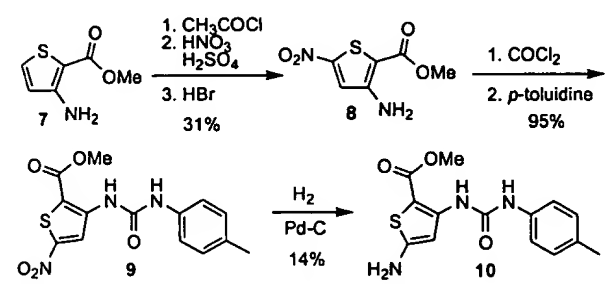
0960-894X/01/$ - see front matter 
PII: S0960-894X(00)00574-6

5-*tert*-butyl-2-thiophene carboxylate was obtained by the condensation of methyl thioglycolate with (*Z*)-3-chloro-4,4-dimethyl-2-pentenenitrile[5] according to a published procedure.[6] Other substituted 4-alkylthiophenes could be prepared by the synthesis of cyanoalkynes, such as nitrile **5** and their subsequent treatment with methyl thioglycolate (Scheme 2).[7]



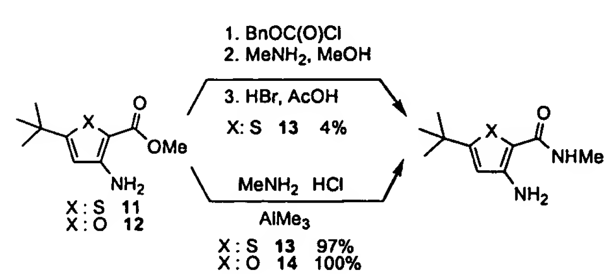

**Scheme 2.** Synthesis of 4-alkylthiophenes from cyanoalkynes.

4-Nitro- and 4-aminothienyl ureas **9** and **10** were obtained from methyl 3-amino-2-thiophenecarboxylate (**7**) via a protection, nitration, and deprotection protocol. The nitro group was reduced after urea formation (Scheme 3).



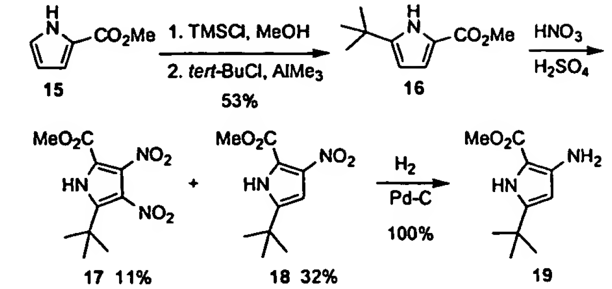

**Scheme 3.** Synthesis of 4-nitro and 4-aminothienyl ureas.

Variation of the ester moiety was studied by Ti(IV) mediated transesterification,[8] or by BOC-protection of the amine, saponification, ester formation and amine deprotection. Amide analogues, such as thiophene **13**, were obtained from the corresponding esters using a Cbz-protection of the amine, amidation and deprotection protocol, or more simply by Weinreb amidation[9] (Scheme 4).



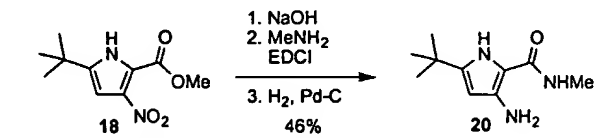

**Scheme 4.** Amidation of esters.

Furyl amines, such as **12**, were synthesized according to a previously published procedure.[10,11] 3-Aminopyrroles were synthesized by Friedel–Crafts alkylation of methyl pyrrole-3-carboxylate (**15**),[12] followed by electrophilic nitration and reduction of the nitro group (Scheme 5).[13]




**Scheme 5.** Synthesis of 3-aminopyrroles.

2-Carbamoyl pyrroles were prepared from ester **18** by saponification and EDCI coupling, followed by reduction of the nitro group (Scheme 6). *N*-Alkyl-3-aminopyrrole was generated by treatment of nitropyrrole **18** with an alkylating agent followed by hydrogenation.



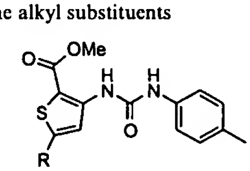

**Scheme 6.** Synthesis of pyrrole amides.

## Results and Discussion

Simple changes in the 5-position of the thiophene ring had a profound effect on potency (Table 1).[14]

Among various alkyl substitutents, *tert*-butyl was optimal (entry **22**). Sterically more demanding groups were not well tolerated (entries **24** and **25**), while smaller alkyl groups also resulted in loss of activity (entry **21**). Surprisingly, urea **10** with an amino group in the 4-position was again a potent inhibitor. Nitrophenyl urea **9** displayed no activity.

**Table 1.** Thiophene alkyl substituents

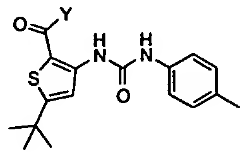



| Entry | R | % Inhibition (5 μM) | p38 α2 $IC_{50}$ (nM) |
|---|---|---|---|
| 21 | *i*Pr | 37 | |
| 22 | $C(CH_3)_3$ | 94 | 413 |
| 23 | 2-Methylpropyl | 0 | |
| 24 | 3-Methylbutyl | 8 | |
| 25 | 1-Hydroxy-1-methylethyl | 34 | |
| 26 | Phenyl | 0 | |
| 27 | 2-Phenylethyl | 17 | |
| 9 | $NO_2$ | 0 | |
| 10 | $NH_2$ | 90 | 441 |

The effect of various ester substitutions is summarized in Table 2. Within the simple alkyl ester series, ana-

logues with bulkier alkyl groups were, in general, weaker inhibitors (entries **28–30**).

**Table 2.** Thiophene ester substituents

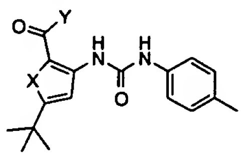


| Entry | Y | p38 α2 $IC_{50}$ (nM) |
|---|---|---|
| **22** | OMe | 413 |
| **28** | OEt | 3020 |
| **29** | OPr | 482 |
| **30** | O*i*Pr | 741 |
| **31** | $O(CH_2)_2OH$ | 57 |
| **32** | $O(CH_2)_3OH$ | 56 |
| **33** | $O(CH_2)_2OCH_3$ | 464 |
| **34** | $OCH_2CO_2CH_3$ | 5310 |

Esters with free hydroxyl groups, such as ureas **31** and **32**, showed a significant increase in potency. Other polar substituents (entries **33** and **34**) led to a significant decrease in activity.

Replacement of the thiophene ring by furan or pyrrole heterocycles resulted in increased potency, except in the case of *N*-methylcarbamoyl pyrroles (Table 3).

**Table 3.** Ester versus amide on various heterocycles

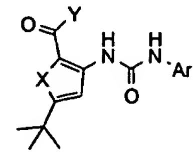


| Entry | X | Y | *E. coli* p38 α2 $IC_{50}$ (nM) |
|---|---|---|---|
| **22** | S | OMe | 248 |
| **35** | S | NHMe | 34 |
| **36** | O | OMe | 73 |
| **37** | O | NHMe | 32 |
| **38** | NH | OMe | 33 |
| **39** | NH | NHMe | 67 |

During further optimization it was found that replacing esters with amides greatly improved the activity of thiophene ureas (Table 3, entry **35** vs **22**). This effect was less significant in the case of furan and pyrrole ureas (entry **37** vs **36** and entry **39** vs **38**). This discrepancy may be due to the thiophene being the most lipophilic of the three heterocycles. The effect of phenyl substitution was first investigated in the thiophene series, followed by a more focused optimization of furan and pyrrole ureas (Table 4).

In the ester series small alkyl groups and halogens were tolerated in the *para* position (Table 3, entry **22**, Table 4, entry **48**). Increasing the size of the *para*-alkyl sub-

**Table 4.** Substitution of the phenyl moiety



| Entry | X | Y | Ar | % Inhibition (0.5 µM) | *E. coli* p38 α2 $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| **40** | S | OMe | Phenyl | 68 | 290 |
| **41** | S | OMe | 4-Ethylphenyl | 40 | 660 |
| **42** | S | NHMe | 4-Ethylphenyl | 70 | 119 |
| **43** | S | OMe | 4-Isopropyl | 10 | |
| **44** | S | NMHe | 4-Isopropyl | 63 | 270 |
| **45** | S | OMe | 4-Phenylphenyl | 6 | |
| **46** | S | OMe | 4-Fluorophenyl | 35 | 830 |
| **47** | S | NHMe | 4-Fluorophenyl | 80 | 88 |
| **48** | S | OMe | 4-Chlorophenyl | 76 | 220 |
| **49** | S | OMe | 4-Aminophenyl | 40 | 750 |
| **50** | S | OMe | 4-Hydroxyphenyl | 44 | 610 |
| **51** | S | OMe | 4-Acetamidophenyl | 23 | |
| **52** | S | OMe | 4-Methoxyphenyl | 8 | |
| **53** | S | OMe | 4-Nitrophenyl | 16 | |
| **54** | S | OMe | 4-Carboxyphenyl | 4 | |
| **55** | S | OMe | 4-Acetylphenyl | 21 | |
| **56** | S | OMe | 2,3-Dichlorophenyl | 79 | 180 |
| **57** | O | OMe | 4-Fluorophenyl | 67 | 210 |
| **58** | O | OMe | 2,3-Dichlorophenyl | 97 | 32 |
| **59** | O | OMe | 3,4- Dichlorophenyl | | 1200 |
| **60** | NH | OMe | Phenyl | | 44 |
| **61** | NH | OMe | 4-Fluorophenyl | 89 | 42 |
| **62** | NH | OMe | 2-Chlorophenyl | 85 | 60 |
| **63** | NH | OMe | 3-Chlorophenyl | 95 | 27 |
| **64** | NH | OMe | 4-Chlorophenyl | 92 | 43 |
| **65** | NH | OMe | 2,3-Dichlorophenyl | 99 | 6 |
| **66** | NH | NHMe | 2,3-Dichlorophenyl | 95 | 44 |
| **67** | NH | OMe | 1-Naphthyl | | 12 |
| **68** | NH | NHMe | 1-Naphthyl | 96 | 28 |
| **69** | NMe | OMe | Phenyl | 32 | 947 |
| **70** | NMe | OMe | 4- Methylphenyl | | 400 |
| **71** | NMe | OMe | 4-Fluorophenyl | 42 | 663 |
| **72** | NMe | OMe | 2,3-Dichlorophenyl | 50 | 387 |
| **73** | NMe | OMe | 1-Naphthyl | 67 | 253 |

stituents led to decreased potency (entries **41**, **43**, and **45**). Placing halogens in both the *ortho* and *meta* positions led to the best inhibitors (entries **56**, **58**, **65**, and **71**). Hydrogen bonding amino and hydroxyl substituents, as in ureas **49** and **50**, caused some loss of activity. Acylation of the amine moiety or alkylation of the phenol led to inactive analogues (entries **51** and **52**, respectively), as did the introduction of electron withdrawing groups other than halogen (entries **53–55**). The overall trend pointed to halogens and small alkyl groups on the phenyl ring to provide optimal lipophilicity. Among the heterocycles, pyrroles consistently showed higher potency.

A few ureas with significant activity against p38 kinase were selected to measure inhibition of IL-6 production in SW1353 cells treated with both cytokines IL-1 and TNF-α.[15] SB 203580 (**1**) was used as a reference compound. The observed cellular activity of our analogues does not directly correlate with the p38 $IC_{50}$ values. However, data presented in Table 5 suggest that functional activity is driven by the combination of primary target potency and appropriate lipophilicity (clogP < 4.5).

Table 5. Inhibition of IL-6 production in SW1353 cells

| Entry | *E. coli* p38 α2 $IC_{50}$ (nM) | Inhibition of IL-6 production $IC_{50}$ (nM) | ClogP (daylight) |
|---|---|---|---|
| **1** | 20 | 50 | 3.6 |
| **22** | 248 | 905 | 5.6 |
| **35** | 34 | 1350 | 4.9 |
| **36** | 73 | 335 | 5.0 |
| **38** | 33 | 1140 | 4.6 |
| **39** | 67 | 15 | 3.4 |
| **61** | 42 | 464 | 4.3 |
| **65** | 6 | 79 | 4.9 |
| **66** | 44 | 16 | 3.8 |
| **67** | 12 | 309 | 5.3 |
| **68** | 28 | 35 | 4.1 |

In conclusion, a thienyl urea series has been identified as potent p38 inhibitors.[16] On exploring different substitution effects, a steep structure–activity correlation was established for the C-5 position of the thiophene ring and for the aryl side of the urea. In addition, furans and pyrroles showed analogous trends. Optimization of the lead thienyl urea **2** led to a 50-fold increase in in vitro activity (compound **65**). The best analogues of this new class show potency in a cellular functional assay of cytokine release.

## Acknowledgements

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New Drugs, Old Drugs

# Tumour necrosis factor inhibitors

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Peter T Nash and Timothy H J Florin

MJA 2005; 183 (4): 205-208



## Abstract

- The cytokine, tumour **necrosis factor**-alpha (TNF-α) plays a key role in the pathogenesis of many chronic inflammatory and rheumatic diseases, in particular, Crohn's disease, rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis.
- Controlled trials have shown that the TNF inhibitors (etanercept, infliximab and adalimumab) significantly reduce symptoms and signs, improve function and quality of life, and reduce radiologically evident damage in patients with rheumatoid diseases. For reasons that are **not** entirely clear, etanercept does **not** work in Crohn's disease.
- Injection site and intravenous reactions and increased risk of infection (in particular, reactivation of tuberculosis) are associated with the use of these agents.

- Increased risk of lymphoproliferative disease, the development of lupus-like syndromes and demyelination, including optic neuritis and reactivation of multiple sclerosis, are under evaluation in long-term follow-up studies.
- The TNF inhibitors are expensive (about $18 000 per year), and in some patients need to be given continuously to maintain benefit, even in the presence of other immunosuppressive therapy.

Although the triggering factors for many autoimmune diseases are **not** known, one of the key inflammatory mediators in the attending chronic inflammatory process is the cytokine, tumour **necrosis factor**-alpha (TNF-α).[1] TNF-α overexpression acts as a driver for inflammation that damages cartilage, bone and bowel mucosa, and TNF-α **inhibition** leads to significant clinical improvements and reduction of this damage. Three TNF-α inhibitors are currently listed by the Pharmaceutical Benefits Scheme (PBS) for use in Australia, in instances of severe disease uncontrolled by other disease modifying measures. They are:

- infliximab — an $IgG_1$ monoclonal antibody (a chimera of human constant and mouse variable regions), for use in rheumatoid arthritis, ankylosing spondylitis and Crohn's disease;
- etanercept — a fusion protein of human IgG and two p75 TNF receptors, for use in rheumatoid arthritis and ankylosing spondylitis (awaiting approval for use in psoriatic arthritis); and
- adalimumab — a humanised $IgG_1$ monoclonal antibody (fully human constant and variable regions), for use in rheumatoid arthritis.

A number of similar agents are under active development. These include a pegylated anti-TNF-α (pegylation adds polyethylene glycol to a protein to prolong its half-life) combined with the p55 receptor for TNF-α.

Infliximab binds to TNF-α and TNF-β and lyses TNF-producing cells to neutralise their activity.[2] It is licensed for use in combination with weekly low-dose methotrexate therapy in rheumatoid arthritis, and is given by intravenous infusion at baseline, 2 weeks, 6 weeks, and thereafter 8-weekly. The dose is 3 mg per kilogram in rheumatoid arthritis and 5 mg per kilogram in ankylosing spondylitis and psoriatic arthritis (Box 1). In Crohn's disease, it is approved for acute and maintenance therapy and the dose is 5 mg per kilogram (Box 1). The presence of murine sequences is associated with the formation of anti-chimeric antibody production, which can result in infusion reactions and a reduction in efficacy with long-term therapy, although the rate of discontinuation

of treatment for this reason is less than 2%.[3]

Etanercept is a recombinant dimer of human TNF receptor proteins fused and bound to human $IgG_1$ that acts competitively to inhibit the binding of TNF to its cell surface receptor. It is given by subcutaneous injection 25 mg twice weekly. Studies have shown that 50 mg given once a week has equal efficacy to twice-weekly injections in patients with rheumatoid arthritis.[4] For reasons that are **not** entirely clear, etanercept is **not effective** in Crohn's disease.

Adalimumab is a monoclonal fully human anti-TNF-$\alpha$ antibody that binds with high affinity to TNF-$\alpha$. It is approved for treating rheumatoid arthritis both in combination with methotrexate and as monotherapy. It is given by subcutaneous injection at a dose of 40 mg every 2 weeks. By replacing murine with human elements, the production of antibodies that neutralise the adalimumab injections is reduced.

## Efficacy

### *Rheumatoid arthritis*

TNF inhibitors are recommended for the treatment of severe and active rheumatoid arthritis after an adequate trial of disease modifying agents (DMARDs) has failed. International consensus guidelines recommend that therapy with two DMARDs in an adequate dosage for an adequate duration (unless **not** tolerated or contraindicated) should be trialled before TNF inhibitors are indicated.[5] TNF inhibitor therapy is expensive (about $18 000 per year), and the PBS authority listing requires the failure of four DMARDs, including methotrexate and three DMARDs used in combination, before therapy for rheumatoid arthritis will be reimbursed. The efficacy and safety of TNF inhibitors was initially demonstrated in rheumatoid arthritis trials where the TNF inhibitor was used as monotherapy; these were followed by combination studies with methotrexate and other disease modifying drugs in severe established disease.[6-10] Subsequent combination trials in patients with early disease showed complete remission rates up to 42% at 2 years of treatment, and prevention of the progression of bone erosion (a surrogate for prevention of irreversible joint damage that leads to deformity and disability) could be shown in 80%.[7] TNF inhibitors have proven efficacy as (i) monotherapy (E1; based on National Health and Medical Research Council levels of evidence[11]); (ii) in combination with methotrexate and with other DMARDs (E1); (iii) added to or replacing pre-existing therapy (E1); (iv) in patients who have **not** previously been treated with methotrexate (E1); and (v) as the first DMARD (E1). Significant improvements are seen in symptoms (especially reduced fatigue and increased energy), signs, function and quality of life.[6-10] Efficacy in juvenile chronic arthritis has also been shown.[12] There is no evidence of superiority of one agent over another (E3),

and failure to respond to one agent does **not** preclude response to another (E3).

### *Psoriatic arthritis*

No conventional DMARD therapy prevents progression of this disease as determined by radiological imaging. However, etanercept and infliximab have been shown to control rash, improve symptoms, quality of life and function, as well as to slow radiologically evident progression in this disease (E2).[13,14] Adalimumab has recently been shown to have similar efficacy.[15] PBS listing for this indication is awaited.

### *Ankylosing spondylitis*

No conventional DMARD therapy has been shown to prevent or reduce radiologically evident progression of this disease. However, randomised controlled trials of etanercept and infliximab as monotherapy have shown their ability to retard radiologically evident progression and significantly reduce symptoms and improve quality of life and function (E2).[16-18]

### *Crohn's disease*

In double-blind randomised placebo-controlled clinical trials, infliximab significantly decreased the Crohn's disease activity index in "treatment-resistant" inflammatory disease, and significantly reduced the number of draining fistulae in fistulating Crohn's disease.[19] Moreover, a study with infliximab is the only randomised placebo-controlled medical treatment trial to ever show improvement in fistulating Crohn's disease.[20] A clinical trial evaluating infliximab in long-term treatment showed it useful for maintaining remission in about 60% of patients with Crohn's disease (E2).[21] The therapy dramatically improves endoscopic disease manifestations, diarrhoeal symptoms and wellbeing. There are promising emerging data for other monoclonal anti-TNF-$\alpha$ therapies, including the pegylated human CDP870 monoclonal antibody[22] and the completely human adalimumab, in the treatment of Crohn's disease,[23] but these drugs are **not** licensed for this indication at present. The recently completed Active Ulcerative Colitis Trial 1 (ACT 1) shows a significant benefit for infliximab in treating severe chronic ulcerative colitis where patients were also taking conventional thiopurine immunosuppression and/or steroids.[24] Safety was a significant issue, with opportunist infection causing one death and an association with three cancers in the active treatment arms.[25]

Many of the "treatment-resistant" cases of Crohn's disease reported in the original and subsequent manufacturer-sponsored trials were in patients treated with steroids, but who had **not** been treated with thiopurine immunosuppression. Such cases would **not**

be termed treatment-resistant in accepted Australian practice where immunosuppression is the normal medical treatment standard. However, the first available biological treatment, infliximab, has certainly transformed the treatment of difficult cases of Crohn's disease. Our review of the early Australian experience with infliximab in Crohn's disease suggests that it has a significant clinical role as an acute adjunctive therapy with conventional thiopurine immunosuppression.[26] This is supported by other more recent retrospective studies.[27,28] There has **not** been a formal, well designed randomised controlled trial of combination TNF inhibitor and conventional immunosuppression in Crohn's disease. This contrasts with rheumatoid arthritis and ulcerative colitis,[24] where trials have either had concomitant conventional immunosuppressive therapy as an inclusion criterion, or stratified patients with treatment-resistant disease who had already been stabilised on conventional immunosuppression. In rheumatoid arthritis in particular, conventional immunosuppression is additive in its effect, both in terms of efficacy and suppressing antibodies to infliximab.[10]

## Adverse effects

TNF inhibitors are generally well tolerated, with prompt onset of action and much earlier relief of symptoms compared with standard DMARD therapy in rheumatoid arthritis, or with standard immunosuppressive therapy in Crohn's disease. Box 2 lists the major adverse effects. Injection site reactions or intravenous infusion reactions of mild to moderate severity occur, and are managed with antihistamines, injection of hydrocortisone or, less commonly, cessation of therapy (E2).[6-10] Serious infections can occur including septic arthritis, infected prostheses and a variety of opportunist infections such as pneumocystis and tuberculosis (E2).[29] In particular, susceptibility to infection and reactivation of latent tuberculosis early after commencement of anti-TNF therapy, and dissemination in a miliary fashion has been documented (E2).[30] This means that patients commencing anti-TNF therapy should have a screening chest x-ray and Mantoux test. However, this guideline was developed primarily for the US and European populations. The interpretation of the Mantoux test in the Australian population, where previous BCG has been common and the prevalence of tuberculosis is low, is difficult. Induration of more than 10 mm and erythema of 15 or greater at 48–72 hours are considered appropriate to avoid clinically irrelevant positive results. Isoniazid therapy for 9 months is indicated if anti-TNF therapy is deemed necessary and the Mantoux result is significantly positive.[24,31] Demyelinating disorders such as reactivation of multiple sclerosis or optic neuritis have been reported.[32] The incidence of lymphoproliferative disease is increased in rheumatoid arthritis, especially in patients with high disease activity, but this also occurs in such patients on methotrexate therapy.[33,34] The TNF inhibitors may increase that risk (E3). Long-term controlled and adequately powered follow-up studies are required to settle this issue. There is no

evidence that TNF inhibitors increase the incidence of other malignancies or recurrence in patients with prior malignancy in the controlled clinical trial database, but further observation in controlled and adequately powered studies are required (E3).[32] The development of antinuclear antibodies and dsDNA antibodies is **not** uncommon, but SLE (systemic lupus erythematosus)-like syndromes are much rarer and abate with drug cessation (E3-2).[32] Other rare reports include pancytopenia, aplastic anaemia,[32] and aggravation of congestive cardiac failure.[32] Safety, apart from anecdotal reports, is unknown in patients with hepatitis B and C infection, and data are limited in pregnancy or lactation.

Box 3 describes situations in which TNF inhibitors are **not** appropriate.

## Conclusion

The TNF inhibitors represent an important new group of agents shown to significantly improve symptoms and signs, function and quality of life, induce remission and reduce objectively measured damage in patients with chronic inflammatory and rheumatic conditions.

In rheumatoid arthritis, there is Level 1[11] evidence for their use as subcutaneous or intravenous injections, generally in combination with methotrexate therapy.

In Crohn's disease, there is accumulating Level 3[11] evidence for their use with conventional immunosuppressive agents. Prospective studies using these drugs in combination therapy are awaited. The results of these studies will be important to ensure rational use of these expensive new therapies.

Toxicity includes injection and infusion reactions, infection risk (particularly with tuberculosis reactivation), and SLE-like syndromes. Risk of lymphoproliferative and demyelinating disease are under ongoing assessment in long-term follow-up studies.

Box 4 contains important messages for patients.

**1 Characteristics of licensed tumour necrosis factor (TNF) inhibitors**

| | Etanercept | Infliximab | Adalimumab |
|---|---|---|---|
| Class | Soluble TNF receptor | TNF-α monoclonal antibody | TNF-α monoclonal antibody |
| Construct | Recombinant fusion protein | Chimeric monoclonal antibody | Human monoclonal antibody |

| | | | |
|---|---|---|---|
| Origin | Entirely human | Human and murine | Entirely human |
| Half-life (days) | 4.8 | 9.5 | 12–14 |
| Use | Effective as monotherapy or in combination with methotrexate therapy | Effective in combination with methotrexate therapy (rheumatoid arthritis) | Effective as monotherapy, or in combination with methotrexate therapy (rheumatoid arthritis) |
| Dosage | | | |
| Rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis | 25 mg subcutaneously twice a week | 3–5 mg/kg intravenously at 0, 2, and 6 weeks; then 4–8 weekly maintenance | 40 mg subcutaneously fortnightly |
| Crohn's disease | Did not show benefit | 5 mg/kg intravenously at 0, 2, and 6 weeks (fistulating); then 8 weekly maintenance | 40 mg subcutaneously fortnightly under evaluation. |

### 2 Major adverse effects of tumour necrosis factor inhibitors

- Injection site and infusion reactions
- Infection — opportunists including fungi and tuberculosis
- Lymphoproliferative disease — non-Hodgkins and Hodgkins lymphoma
- Demyelinating disease — reactivation of multiple sclerosis and optic neuritis
- SLE-like syndromes
- Aggravation of congestive cardiac failure

SLE = systemic lupus erythematosus.

### 3 Situations in which anti-tumour necrosis factor therapy is considered inappropriate for safety reasons[32]

- After previous tuberculosis (except after a full course of modern anti-tuberculosis therapy, ongoing isoniazid cover and the patient being made aware of the risks and benefits)

- Within 12 months of septic arthritis
- Patients with an infected prosthesis
- Patients with recurrent chest infections or bronchiectasis
- Patients with indwelling urinary catheters
- Patients with multiple sclerosis or demyelinating illness
- Within 10 years of any malignancy (apart from fully resected basal cell carcinoma more than 5 years before)
- During pregnancy and lactation
- Patients with congestive cardiac failure
- Patients with chronic cutaneous ulceration, but **not** pyoderma gangrenosum

**4 Important messages for patients**

- Tumour **necrosis factor** (TNF) inhibitors offer major therapeutic gain, which can revolutionise quality of life and stop damage in selected patients
- These drugs are very expensive
- Side effects are rare, but can be serious (eg, decreased immunity to infections)
- When and how to use these drugs, and with what other medications, is under active study
- The long-term safety of TNF inhibitors is being evaluated

## Competing interests

P N has received research grants for clinical trials and has lectured on behalf of, or consulted for, Wyeth, Abbott and Schering-Plough. T F is involved with clinical trials and has served on an industry advisory board for Schering-Plough.

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# Phase I-II trial of a monoclonal anti-tumor necrosis factor alpha antibody for the treatment of refractory severe acute graft-versus-host disease

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In a multicenter pilot study, 19 patients with severe acute graft- versus-host disease (aGVHD) refractory to conventional therapy and serotherapy with a monoclonal anti-interleukin-2 receptor antibody were treated by in vivo infusion of a monoclonal anti-tumor necrosis factor alpha (TNF alpha) antibody (B-C7). Ten patients were grafted from a genotypically identical sibling, five from an HLA-mismatched family member, and four from an HLA-matched unrelated donor. Before B-C7 treatment, 15 patients had grade IV and four had grade III GVHD. In all cases, patients received cyclosporine/methotrexate as aGVHD prophylaxis. Patients were administered increasing doses of antibody (from 0.1 to 0.4 mg/kg). The antibody was infused in bolus daily for 4 days and then every other day twice (6 doses). No side effects were observed during treatment regardless of the dose level used. Changes in peripheral blood cell counts occurred in 8 of the 19 patients and appeared to be unrelated to B-C7. No truly complete response was observed; eight patients achieved a very good partial response (42.6%) and six a partial response (31.5%). The treatment was ineffective in five patients (26.4%). When present, the response occurred early (less than 3 days). In the 14 responding patients, gut lesions responded best (100%), followed by skin (85%) and liver (35.7%) lesions. In 9 of 11 evaluable patients (81%), GVHD recurred when treatment was discontinued in a median delay of 3 days (range, 2 to 120 days). All except one died from aGVHD. Two patients did not experience GVHD recurrence and are still alive 13 and 18 months post-bone marrow transplantation. This pilot study shows that a monoclonal anti-TNF alpha antibody may be of benefit to some patients with severe refractory aGVHD, but is ineffective to prevent GVHD recurrence in the majority of cases.

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